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(54) Title: SOLID MYCOCTOXIN CARRIERS

(57) Abstract: The invention relates to a method for binding mycotoxins to a solid carrier, comprising the following steps: contacting a mycotoxin containing solution, suspension or aerosol with a mycotoxin imprinted polymer, separating the bound mycotoxin from said solution, suspension or aerosol and optionally separating the mycotoxin from said polymer.

Solid Mycoctoxin Carriers

The invention relates to a method for binding mycotoxins to a solid carrier.

Contamination of agricultural products by moulds has been observed for centuries posing a substantial hazard to the health of both, humans and animals (Pittet 1998). Among the numerous acute toxic, carcinogenic, mutagenic, teratogenic, and estrogenic secondary metabolites, deoxynivalenol (DON) and zearalenone (ZON) are prominent representatives. DON and ZON are the most relevant mycotoxins produced by Fusarium species, with respect to the agricultural production in central Europe. The pathological pathway of DON (12,13-Epoxy-3a,7a,15-trihydroxy-trichothec-9-en-8-one) is based on the epoxy group attached to the trichothecen ring (between C12 and C13) and the position and structure of its side groups (Binder et al. 1998). ZON is chemically described as [(-)-(3S,11E)-3,4,5,6,9,10-Hexahydro-14,16-dihydroxy-3-methyl-1H-2-benzoxa-cyclotetradecin-1,7(8H)-dion] and belongs to the group of resorcyclic acid lactones. An extensive review of the occurrence and toxicity of deoxynivalenol and zearalenone including risk assessment was conducted in Canada in 1987 by Scott and co-workers (Kuiper-Goodman et al. 1987). A global study has been published by Tanaka et al. (Tanaka et al. 1988).

Generally, the standard procedures for the determination of mycotoxins involve discontinuously operated laboratory methods. The common scheme for the analysis of mycotoxins is based on an extraction step followed by a time consuming clean-up procedure with a non-polar solvent and subsequent solid-phase extraction using e.g. an immunoaffinity column (Scott et al. 1993; Won-Bo et al. 1997). Thus obtained extracts are then pre-concentrated and finally derivatised for the actual analysis using thin layer chromatography (TLC), liquid chromatography (LC, HPLC) or gas-chromatographic methods such as GC/MS. The increased occurrence of DON in commercial beers resulted in enhanced efforts to develop simpler GC/MS methods involving ethyl acetate extraction and direct analysis of the extract (Hastings and Steenroos 1995; Schwarz et al. 1995). Enzyme-linked immunosorbent assays (ELISA)

have been used for direct mycotoxin detection in beer with reasonable success. After clean-up of the extract good correlation to the previously mentioned methods has been achieved (Scott et al. 1997). Despite the gain in analysis time, ELISA tests have to be considered as cost intensive screening method and not suitable for decontaminating mycotoxin contaminated material on an industrial scale. Indeed, Jodlbauer et al. (2002) reported that direct imprinting (= using the target molecule as template during the imprinting procedure) of the mycoctoxins (ochratoxin A (OTA) is not possible, especially due to the "particularly problematic" residual template (e.g. for trace analysis).

In many analytical problems the detection of small traces of substances relies on the existence of molecular recognition elements capable of binding analytes with high affinity and selectivity. Molecular recognition elements which are therefore typically used are proteins like polyclonal, monoclonal or recombinant antibodies, enzymes, receptors, or even oligo-nucleotides. Besides their main advantage of high selectivity and association constants for the interaction process, they all have significant disadvantages: (i) they are more or less difficult to obtain therefore being expensive, (ii) they only work in a certain temperature range, (iii) generally in aqueous buffers and (iv) not under harsh conditions. In addition to their poor stability they are difficult to regenerate after one binding event. Moreover, specificity to the desired target analytes cannot always be generated due to natural limitations: generation of antibodies to antigens is frequently difficult owing to the small molecular mass and limited chemical complexity. Even though boosting immunizations increase the probability of developing high affinity antibodies, some substances are too toxic causing the death of the animal before immunization can take place.

Accordingly there is a need for alternative methods for mycotoxin binding, especially for mycotoxin decontamination and mycotoxin analytics. The method should preferably be robust (i.e. suitable for field testing without the necessity of having a laboratory facility performing such tests or methods), specific, reliable, re-useable, and useable on an industrial scale.

The present invention therefore provides a method for binding mycotoxins to a solid carrier, comprising the following steps:
- contacting a mycotoxin containing solution, suspension or aerosol with a mycotoxin imprinted polymer,
- separating the bound mycotoxin from said solution, suspension or aerosol and
- optionally separating the mycotoxin from said polymer.

According to the present invention methods have been established to successfully provide mycotoxin imprinted polymers. These polymers have – despite the high degree of uncertainty being present in the field of imprinting polymers against complex organic structures, such as mycotoxins, in a specific way - been proven to be a suitable alternative for anti-mycotoxin antibodies, both in analytical processes and especially on an industrial scale e.g. for decontaminating mycotoxin-contaminated material.

In comparison with the methods being available in the art for detecting and binding mycotoxins, the present method provides significant advantages, especially with respect to antibodies or antibody-derived solid carriers: the storage stability of the present mycotoxin imprinted polymers exceeds 3 years, whereas antibodies are storeable for 6 month only (as a maximum). Temperature stability is significantly enhanced with the present polymers, which makes them extremely suitable for industrial methods where high temperature steps (e.g. for pathogen de-contamination) have to be applied. The present polymers are by far more resistant to (organic) solvents or against low or high pH (2-11, compared to 5 to 8 for antibodies).

The present polymers have also been proven to be easily producible with surprisingly high reproducibility. Automation of the production methods for the present polymers is manageable without much effort – again, in contrast to the difficult upscaling and automation of antibody-based techniques.

Specifially in view of the paper by Jodlbauer et al. (2002) it was surprising that for specific macotoxin being stucturally

different from ochratoxin A (OTA) and its related structures27(such as ochratoxin A, ochratoxin B, ochratoxin C, ochratroxin A-ME, ochratoxin B-ME, ochratoxin B-Et, ochratoxin alpha, ochratoxin beta (as described in Fig. 1 of Jodlbauer et al. (2002), especially for DON, 20N and their structural and chemical analogues (trichothecene and resorcydic acid lactones, the application of the (direct) molecular imprinting technology is practically working on an industrial scale even in trace analysis. With the present invention it was demonstrated with HPLC and SPE results that for these mycotoxins, especially for trichothecene and resorcyclic acid (actones; preferably DON and ZON) direct imprinting is possible. It could be demonstrated that even after direct impriting with the target molecule SPE matrices for DON, which show improvied retention of DON can be obtained in a properly usable form. These developed polymers according to the present invention are suitable as solid phase extraction material for the extraction of the mycotoxin according to the present invention, especially for deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ac-DON), and zearalenone (ZON) from standard solutions. Such extraction material did not show bleeding events which would hinder the application of this matrix material on an industrial scale, even in food technology (e.g. for beer production). For example, DON and 3-acetyl-DON recovery rates from the SPE of 87.5 and 94.5 % have been achieved, respectively, after solid phase extraction with the imprinted polymers from aqueous standard solutions.

Recovery rates in indirect imprinting of up to 94.5 % confirm that template bleeding does not falsify the results in the selected direct imprinting approach.

The present polymers may also be re-used 100 times or more (depending on the specific use) for one year or even longer, whereas antibody-based methods may be used only once, at least with the initial quality criteria.

With respect to the other detection methods for mycotoxins available in the art (liquid phase extraction, GC, LC, HPLC,...), the present method is superior i.a. with respect to its specificity (i.e. comparable with antibody specificity),

simplicity and usability on industrial scale, e.g. for food industry, beverage industry, homeland security, etc..

Preferably, the mycotoxin imprinted polymer is specifically imprinted to a template selected from the group comprising calonectrin, deacetylcalonectrin, 7alpha,8alpha-dihydroxy-calonectrin, 7-hydroxycalonectrin, 8-hydroxy-calonectrin, 3-acetyldeoxy-nevalenol, 15-acetoxydeoxy-nivalenol, 3-acetyl-4,7-deoxynivalen, 3,15-diacetyl-deoxynivalenol, 4,7-dideoxynivalenol, deoxynivalenol, fusarenon-X, nivalenol, diacetylnivalenol, 4-acetyl-scirendiol, diepoxy-diacetyl-scirpenol, 4,15-diacetylscirpendiol, 3,4-diacetylscirpenol, 3,15-diacetylscirpenol, 4,15-diacetylscirpenol, 4,15 -diacetylscirpentriol, 7-hydroxy-isotrichodermin, 8-hydroxy-isotrichodermin, isotrichodermin, 3-monoacetylscirpenol, 15-monoacetylscirpenol, 8-oxodiacetylscirpenol, scirpentriol, triacetylscirpen, 15-acetyl-T-2-tetraol, acetyl T-2 toxin, 3,15-diacetyl-7,8-dihydroxy-12,13-epoxytrichotec-9-en, HT-2, 3'-hydroxy-HT-2, 3-hydroxytrichothecen, 3'-hydroxy-T-2-triol, neosolaniol, neosolaniol-monoacetat, NT-1 toxin, NT-2 toxin, 4-propanoyl HT-2, sporotrichiol, 4,8,15-triacetylscirpendiol, T-2 tetraol, T-2 toxin, 6'8'-dihydroxyzearalen, 5-formylzearalenon, 3'-hydroxyzearalenon, 7'-dehydrozearalenon, 8'-epi-hydroxyzearalenon, 8'-hydroxyzearalenon, zearalanon, zearalanol, zearalenol - cis, alpha,beta,zearalenol - trans, zearalenon-F2, butenolid, FS-1, FS-2, fusarin C, fusariocin C, fusarochromanon, 4-ipomeanol, moniliformin, poaefusarin, sporofusarin, trichotriol, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, aflatoxin M2, 4R-hydroxyochratoxin A, 4S-hydroxyochratoxin A, 10-hydroxyochratoxin A, fumonisin B1, fumonisin B2, fumonisin B3 or mixtures thereof.

The mycotoxin containing solution, suspension or aerosols may be any solution, suspension or aerosol being contactable with an imprinted polymer. Even gas containing preparations with mycotoxins may be regarded as such solutions or suspensions. Preferred solutions or suspensions to be contacted with the imprinted polymers according to the present invention are those common in the field of mycotoxin analytics and decontamination. Therefore, these solutions or suspensions are preferably selec-

ted from the group comprising field crop preparations, food preparations, water preparations, especially waste water, rinsing water, food processing water, environmental or industrial water samples or in the atmosphere or air samples/aerosols.

Although the skilled man in the art can apply the teachings of the present invention with the knowledge in the art of polymer imprinting to different polymers being built up by many different functional monomers, it is preferred for the present invention that the mycotoxin imprinted polymers are based on functional monomers selected from the group comprising 4-vinylpyridine (4-VP), methacrylic acid (MAA), 2-trifluoromethylacrylic acid (TFM), methylmethacrylates or other alkylmethacrylates, alkylacrylates, allyl or arylacrylates and methacrylate, cyanoacrylate, styrene, alpha-methylstyrene, vinylester, especially vinylacetate, vinylchloride, methylvinylketon, vinylidenchloride, acrylamide, methacrylamid, acrylonitril, methacrylonitril, 2-acetamidoacrylic acid, 2-(acetoxyacetoxy)ethylmethacrylate, 1-acetoxy-1,3-butadiene, 2-acetoxy-3-butenenitrile, 4-acetoxystyrene, acrolein, acroleindiethylacetal, acroleindimethylacetal, acrylamide, 2-acrylamidoglycolic acid, 2-acrylamido-2-methylpropansulfonic acid, acrylic acid, acrylanhydride, acrylonitrile, acryloylchloride, (R)-alpha-acryloxy-beta, beta'-dimethyl-g-butyrolactone, N-acryloxysuccinimide, N-acryloxytri.s(hydroxymethyl) aminomethane, N-acryloylchloride, N-acryloylpyrrolidone, N-acryloyltris(hydroxymethyl) amino methane, 2-amino ethyl methacrylate, N-(3-aminopropyl)methacrylamide, (o,m,or p)-amino-styrene, t-amylmethacrylate, 2-(1-aziridinyl)ethylmethacrylate, 2,2'-azobis-(2-amidinopropane), 2,2'-azobisisobutyronitrile, 4,4'-azobis-(4-cyanovaleric acid), 1,1'-azobis-(cyclohexanecarbonitrile);2,2'azobis-(2,4-dimethylvaleronitrile); 4-benzyloxy-3-methoxystyrene;2-bromoacrylic acid; 4-bromo-1-butene; 3-bromo-3,3-difluoropropane; 6-bromo-1-hexene; 3-bromo-2-methacrylonitrile: 2-(bromomethyl)acrylic acid; 8-bromo-1-octene; 5-bromo-1-pentene; cis-1-bromo-1-propene;.beta.-bromostyrene;p-bromostyrene, bromotrifluoro ethylene; (+/-)-3-buten-2-ol;1,3-butadiene;1,3-butadiene-1,4-dicarboxylic acid; 3-butenal diethyl acetal; 1-butene; 3-buten-2-ol; 3-butenyl chloroformate; 2-butylacrolein;

N-t-butylacrylamide;butyl acrylate; butyl methacrylate; (o,m,p)-bromostyrene; t-butyl acrylate; (R)-carvone; (S)-carvone; (-)-carvyl acetate; cis 3-chloroacrylic acid; 2-chloroacrylonitrile; 2-chloroethyl vinyl ether; 2-chloromethyl-3-trimethylsilyl-1-propene; 3-chloro-1-butene; 3-chloro-2-chloromethyl-1-propene; 3-chloro-2-methyl propene; 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; 3-chloro-1-phenyl-1-propene; m-chlorostyrene; o-chlorostyrene; p-chlorostyrene; 1-cyanovinyl acetate; 1-cyclopropyl-1-(trimethylsiloxy)ethylene; 2,3-dichloro-1-propene; 2,6-dichlorostyrene; 1,3-dichloropropene; 2,4-diethyl-2,6-heptadienal; 1,9-decadiene; 1-decene; 1,2-dibromoethylene; 1,1-dichloro-2,2-difluoroethylene; 1,1-dichloropropene; 2,6-difluorostyrene; dihydrocarveol; (+/-)-dihydrocarvone; (-)-dihydrocarvyl acetate; 3,3-dimethylacrylaldehyde; N,N'-dimethylacrylamide; 3,3-dimethylacrylic acid; 3,3-dimethylacryloyl chloride; 2,3-dimethyl-1-butene; 3,3-dimethyl-1-butene; 2-dimethyl aminoethyl methacrylate; 2,4-dimethyl-2,6-heptadien-1-ol; 2,4-dimethyl-2,6-heptadienal; 2,5-dimethyl-1,5-hexadiene; 2,4-dimethyl-1,3-pentadiene; 2,2-dimethyl-4-pentenal; 2,4-dimethylstyrene; 2,5-dimethylstyrene; 3,4-dimethylstyrene; divinyl benzene; divinyltetramethyl disiloxane; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-porphine; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-propionic acid; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-propionic acid disodium salt; 3,9-divinyl-2,4,8,10-tetraoraspiro[5,5]undecane; divinyl tin dichloride; 1-dodecene; 3,4-epoxy-1-butene; 2-ethyl acrolein; ethyl acrylate; 2-ethyl-1-butene; (+/-)-2-ethylhexyl acrylate; (+/-)-2-ethylhexyl methacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol triacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate; ethyl methacrylate; ethyl vinyl ether; ethyl vinyl ketone; ethyl vinyl sulfone; (1-ethylvinyl)tributyl tin; m-fluorostyrene; o-fluorostyrene; p-fluorostyrene; glycol metacrylate (hydroxyethyl methacrylate); GA GMA; 1,6-heptadiene; 1,6-heptadienoic acid; 1,6-heptadien-4-ol; 1-heptene; 1-hexen-3-ol; 1-hexene; hexafluoropropene; 1,6-hexanediol diacrylate; 1-hexadecene; 1,5-hexadien-3,4-diol; 1,4-hexadiene; 1,5-hexadien-3-ol; 1,3,5-hexatriene; 5-hexen-1,2-diol; 5-hexen-1-ol; hydroxypropyl acrylate; 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene; isoamyl methacrylate; isobutyl methacrylate; isoprene; 2-isopropenylaniline; isopropenyl chloroformate; 4,4'-isopropylidene

dimethacrylate; 3-isopropyl-alpha-alpha-dimethylbenzene isocyanate; isopulegol; itaconic acid; itaconalyl chloride; lead(II)acrylate; (+/-)-linalool; linalyl acetate; p-mentha-1,8-diene; p-mentha-6,8-diene-2-ol; methyleneamino acetonitrile; methacrolein; [3-(methacryloylamino)-propyl]trimethylammonium chloride; methacrylamide; methacrylic acid; methacrylic anhydride; methacrylonitrile; methacryloyl chloride; 2-(methacryloyloxy)ethyl acetoacetate; (3-methacryloxypropyl)trimethoxy silane; 2-(methacryloxy)ethyl trimethyl ammonium methylsulfate; 2-methoxy propene (isopropenyl methyl ether; methyl-2-(bromomethyl)acrylate; 5-methyl-5-hexen-2-one; methyl methacrylate; N,N'-methylene bisacrylamide; 2-methylene glutaronitrile; 2-methylene-1,3-propanediol; 3-methyl-1,2,-butadiene; 2-methyl-1-butene; 3-methyl-1-butene; 3-methyl-1-buten-1-ol; 2-methyl-1-buten-3-yne; 2-methyl-1,5-heptadiene; 2-methyl-1-heptene; 2-methyl-1-hexene; 3-methyl-1,3-pentadiene; 2-methyl-1,4-pentadiene; (+/-)-3-methyl-1-pentene; (+/-)-4-methyl-1-pentene; (+/-)-3-methyl-1-penten-3-ol; 2-methyl-1-pentene; .alpha.-methyl styrene; t-alpha-methylstyrene; t-beta.-methylstyrene; 3-methylstyrene; methyl vinyl ether; methyl vinyl ketone; methyl-2-vinyloxirane; 4-methylstyrene; methyl vinyl sulfone; 4-methyl-15-vinylthiazole; myrcene; t.beta.-nitrostyrene; 3-nitrostyrene; 1-nonadecene; 1,8-nonadiene; 1-octadecene; 1,7-octadiene; 7-octene-1,2-diol; 1-octene; 1-octen-3-ol; 1-pentadecene; 1-pentene; 1-penten-3-ol; t-2,4-pentenoic acid; 1,3-pentadiene; 1,4-pentadiene; 1,4-pentadien-3-ol; 4-penten-1-ol; 4-penten-2-ol; 4-phenyl-1-butene; phenyl vinyl sulfide; phenyl vinyl sulfonate; 2-propene-1-sulfonic acid sodium salt; phenyl vinyl sulfoxide; 1-phenyl-1-(trimethylsiloxy)ethylene; propene; salfrole; styrene (vinyl benzene); 4-styrene sulfonic acid sodium salt; Styrene sulfonyl chloride; 3-sulfopropyl acrylate potassium salt; 3-sulfopropyl methacrylate sodium salt; tetrachloroethylene; tetracyano ethylene; tetramethyldivinyl siloxane; trans 3-chloroacrylic acid; 2-trifluoromethyl propene; 2-(trifluoromethyl)propenoic acid; 2,4,4'-trimethyl-1-pentene; 3,5-bis(trifluoromethyl)styrene; 2,3-bis(trimethylsiloxy)-1,3-butadiene; 1-undecene; vinyl acetate; vinyl acetic acid; 4-vinyl anisole; 9-vinyl anthracene; vinyl behenate; vinyl benzoate; vinyl benzyl acetate; vinyl benzyl alcohol; 3-vinyl benzyl chloride; 3-(vinyl benzyl)-2-chloro-

ethyl sulfone; 4-(vinyl benzyl)-2-chloroethyl sulfone; N-(p-vinyl benzyl)-N,N'-dimethyl amine; 4-vinyl biphenyl (4-phenyl styrene); vinyl bromide; 2-vinyl butane; vinyl butyl ether; 9-vinyl carbazole; vinyl carbinol; vinyl cetyl ether; vinyl chloroacetate; vinyl chloroformate; vinyl crotanoate; vinyl cyclohexane; 4-vinyl-1-cyclohexene; 4-vinylcyclohexene dioxide; vinyl cyclopentene; vinyl dimethylchlorosilane; vinyl dimethylethoxysilane; vinyl diphenylphosphine; vinyl 2-ethyl hexanoate; vinyl 2-ethylhexyl ether; vinyl ether ketone; vinyl ethylene; vinyl ethylene iron tricarbonyl; vinyl ferrocene; vinyl formate; vinyl hexadecyl ether; vinylidene fluoride; 1-vinyl imidizole; vinyl iodide; vinyl laurate; vinyl magnesium bromide;vinyl mesitylene; vinyl 2-methoxy ethyl ether; vinyl methyl dichlorosilane; vinyl methyl ether; vinyl methyl ketone; 2-vinyl naphthalene; 5-vinyl-2-norbornene; vinyl pelargonate; vinyl phenyl acetate; vinyl phosphonic acid, bis(2-chloroethyl)ester; vinyl propionate; 4-vinyl pyridine; 2-vinyl pyridine; 1-vinyl-2-pyrrolidinone; 2-vinyl quinoline; 1-vinyl silatrane; vinyl sulfone; vinyl sulfone (divinylsulfone); vinyl sulfonic acid sodium salt; o-vinyl toluene; p-vinyl toluene; vinyl triacetoxysilane; vinyl tributyl tin; vinyl trichloride; vinyl trichlorosilane; vinyl trichlorosilane (trichlorovinylsilane); vinyl triethoxysilane; vinyl triethylsilane; vinyl trifluoroacetate; vinyl trimethoxy silane; vinyl trimethyl nonylether; vinyl trimethyl silane; vinyl triphenylphosphonium bromide (triphenyl vinyl phosphonium bromide); vinyl tris-(2-methoxyethoxy)silane; vinyl 2-valerate; acrylate-terminated or otherwise unsaturated urethanes, carbonates, and epoxies, especially allyl diglycol carbonate, glycidyl acrylate, glycidyl methacrylate, allyl glycidyl ether, and 1,2-epoxy-3-allyl propane; or mixtures thereof.

According to another aspect, the present invention also relates to a method for preparing a mycotoxin imprinted polymer comprising the following steps:

- providing a mycotoxin template with a functional monomer in combination with a porogenic solvent so that a prepolymerisation complex of said template with said functional monomer is formed,
- co-polymerising said functional monomer with a crosslinker so that a co-polymer is formed,

- removing said template from said formed co-polymer at least partially and
- optionally further processing said formed co-polymer by a size reduction method, especially by crushing, grounding, milling or combinations of such size reduction methods.

The technique of imprinting polymers is per se known in the art for about 20 years. In 1981 Mosbach and coworkers have shown that the "non-covalent" imprinting protocol produced highly selective molecularly imprinted polymers (MIPs) with basic laboratory instrumentation (Arshady et al., 1981; US patent 4,406,792). Molecularly imprinted polyacrylic polymers generated by the non-covalent imprinting method are mechanically and thermally stable, and resistant to all kind of influences like heat, pressure, time, and basic or acidic chemicals.

Different forms of molecularly imprinted polymers and examples of the conventional bulk polymerisation and the more innovative microspheres polymerisation exist. However, the art of providing imprinted polymers is still unpredictable with respect to the principle availability of polymers imprinted to a specific compound. Since the polymers are usually made in an organic solvent, the application of the polymers in aqueous media may lead to changes in the strenght of interaction between substrate and the polymer. The polymer could also show different three dimensional structures in aqueous media due to shrinking or swelling and thereby altering the size of the compartments of the polymer.

Protic solvents, such as water and methanol have been regarded as hindering polymerisation and disrupting the template-monomer hydrogen-bonding interactions being the prerequisite for providing a suitable imprinted polymer (Norrlöw et al, 1984).

Wulff and co-workers developed polymerisable derivatives of the template molecule, which are co-polymerised with a cross-linking monomer (Wulff, 1995). These derivatives are obtained by forming covalent bonds between the template and suitable polymerisable monomers. In order to remove the template from the polymer and liberate the binding sites, these covalent bonds have to be

chemically cleaved, and are subsequently re-formed during rebinding of the target molecule. Mosbach and co-workers have followed a different approach: they rely on the formation of a prepolymerisation complex between monomers carrying suitable functional groups and the template through non-covalent bonds, such as ionic interactions or hydrogen bonding (Mosbach et al, 1996). Following polymerisation, the functional groups are held in position by the polymeric network, whereas the template can be simply removed by solvent extraction. The principle means of rebinding the "antigen" to these polymers is again through non-covalent interactions. This self-assembly process is more similar to the natural recognition process, since most biomolecular interactions are non-covalent in nature. In summary, there are following approaches to obtain a molecularly imprinted polymer:

1. The template is bound by metal complexation to the monomer/polymer ligands like in Immobilized Metal Affinity Chromatography (IMAC). Using this coordinative binding approch (e.g. binding with copper complexes to compounds containing imidazole) there is the possibility to tune the affinity of the binding site by substitution of the metal ion (Matsui et al, 1996).

2. Covalent molecular imprinting: The print molecule is coupled to a vinyl monomer by means of a reversible covalent linkage and the derivatised print molecule then copolymerised with an excess amount of cross-linking agent. The print molecule is then cleaved from the imprinted sites using chemical methods. Rebinding of the print molecules requires the original reversible bond to reform under conditions that favour substrate uptake. Most chemical linkages in the covalent approach are boronic esters, Schiff bases, and ketals. Binding sites created by covalent binding are characterised by homogeneity in binding strength throughout the whole polymer but have the major disadvantage of slow binding kinetics. In addition, it is not always possible to form covalent bonds between print molecules and functional monomers due to the lack of functionality on the print molecule.

3. Self-assembly of the functional monomer and the template by non-covalent electrostatic interaction or hydrogen bonding and subsequent co-polymerisation with the cross-linked monomer: ion-

ic interactions, hydrogen bonding interactions and hydrophobic effects can all be employed simultaneously. Eluting the template by a washing step generates the binding sites. Since non-covalent molecular interactions dominate our living world, their use in molecular imprinting is in principle unlimited. Nevertheless, the non-covalent approach has disadvantages. Due to the excess of functional monomers used to facilitate the formation of pre-polymerisation complexes (print molecule with functional monomer), a large number of unspecific binding sites are created. Moreover, for those functional monomers that do interact with the print molecule during the pre-arrangement, their final distribution and orientation are not always perfectly ensured due to molecular motions during polymerisation. This is partly the reason for the heterogeneity of binding sites in non-covalently imprinted polymers. Therefore, non-covalently imprinted polymers are often compared with polyclonal antibodies. Through competitive radioligand binding analysis, this feature has been studied by following a two binding site model (Andersson et al, 1995). Non-covalent imprinting is especially preferred according to the present invention.

4. The semi-covalent (sacrificial) molecular imprinting: In this method developed by Sellergren and Andersson (Sellergren et al, 1990) the template binds covalently e.g. ester bond formation to the functional monomer during imprinting. After the imprinting process the esters are hydrolysed. During the following reaction the acid group of the functional monomer reacts to CO_2 and the alkyl residue. The subsequent rebinding takes place by non-covalent interactions. In general the semi-covalent molecular imprinting approach should combine the homogeneity of binding sites from the covalent approach with the equilibrium of the substrate rebinding from the non-covalent approach. In fact, the same limitations that are encountered in the covalent approach (chemical synthesis and cleavage) still prevent the general applicability of this method. Moreover, removal of the sacrificial spacer most likely interferes with the spatial arrangement of the functional groups at the polymer and the substrate and is therefore less selective.

5. Bio-imprinting or protein imprinting utilizes the ability of

proteins to form specific binding interactions to an unnatural substrate tailored by a bioimprinting step. Conventionally imprinted polymers are highly cross-linked synthetic polymers, usually linear copolymers obtained through ordinary radical polymerisation. In contrast, proteins consist of a primary amino acid sequence forming the ternary structure of globular proteins. Three-dimensional structures are mainly stabilised by concerted, multiple, weak interactions, including hydrogen bonds, ionic forces, and hydrophobic effects. A large variety of proteins e.g. subtilisin, a protease, and bovine serum albumin (BSA) were allowed to interact with a template in aqueous solution as reported by Klibanov et al.(Dabulis et al, 1992). After freeze drying the template was removed by subsequent washing with anhydrous organic solvent. The resulting templated proteins were found to be several times more active than the native protein in anhydrous media due to shape-selective and enantioselective molecular recognition characteristics for the substrate. Alternatively, Keyes et al. prepared semi-synthetic protein imprints with altered catalytic activities (Albert et al., 1992).

6. Surface imprinting of microorganisms is one of the most recent techniques emerged from molecular imprinting. Three immediate issues have to be addressed hereby: (1) to decide on appropriate functionality in the sites, (2) to find a way of reproducing the size and shape of a microorganism in the polymer matrix, and (3) to position the functionality within the defined sites. It is also important to render the rest of the surface non-functional or inert and to ensure that recognition sites are easily accessible. It is obvious that this clearly requires the development of appropriate methods or techniques and special polymerisable monomers, such as 2-aminophenylboronic acid or plasma deposited hexafluoropropylene. To date, there have been only few publications in this area (Bossi et al, 2001; Shi et al, 1999).

In some cases self-assembly of the template and the functional monomer has yielded molecular imprinted polymers with rebinding affinities and selectivity comparable to antibody-antigen interactions, however, there is a high unpredictability in the art so that it is not forseeable whether a given template may result in

an efficient and selective imprinted polymer. Moreover providing the polymer may also be dependent on specific choice of selective reaction partners (functional monomers, templates, cross-linkers, reaction starters, their relative ratios, etc.) and reaction parameters, the optimal nature of which is not predictable by the man skilled in the art.

As mentioned above, non-covalent molecular imprinting is the preferred method for providing the mycotoxin imprinted polymers according to the present invention.

Optimisation of the polymer structure, however, is a rather complicated process in non-covalent molecular imprinting. On the one hand, the polymers should be rigid enough to preserve the structure of the cavity after removal of the template. On the other hand, the polymer should be highly flexible to facilitate equilibrium between release and re-uptake of the template in the cavity. Furthermore, accessibility of as many cavities as possible is required as well as high thermal and mechanical stability (macro porous polymers with a high inner surface area). Polymer shrinking in organic or aqueous media should be as low as possible. In addition, the polymer rigidity can be crucial in the preparation of chromatographic stationary phases and surface layers e.g. for ATR crystals.

(a) The print molecule or template: In general, a print molecule should be soluble in organic solvents and provide suitable functional groups for interaction with the functional monomers, to ensure stable complexation. The structure and chemical characteristics of the template usually determines the nature of the imprinting approach. Functional monomers are generally selected for strong interactions with the template. So far there is no general protocol or rational design scheme aiding the design of molecularly imprinted polymers (MIPs). Hence, only empirical knowledge is the starting point for the decision which ingredients should be selected. For example: When the print molecule can form complexes with certain metal ions, metal chelating functional monomers may be suitable. Methacrylic acid can provide ionic interactions to basic functional groups within the template. If hydrobhobic interactions are desired, the imprint-

ing solvent can be adjusted accordingly to enhance the binding strengths.

(b) Functional monomer: It is of importance that the functional monomer strongly interacts with the template prior to polymerisation, since the structure of the resulting assemblies in solution presumably defines the subsequently formed binding sites. By stabilizing the monomer-template assemblies it is possible to increase the number of imprinted sites. At the same time the number of non-specific binding sites will be minimized. The functional monomer should interact with the template and form e.g. hydrogen bridges or non-covalent interactions. Frequently, acrylate and derivatives (acrylamide, methacrylate, etc) or styrene is used as functional monomer. Polyacrylate-based matrices have the advantage that the polymerisation is easy to initiate (UV-light, heat) and finalized within hours. Besides, the solubility is of crucial importance for the practical usefulness of the functional monomer. Acrylates are soluble in a wide range of solvents and under an ambient temperature and pressure.

The most widely used functional monomer is methacrylic acid. The carboxylic acid group can form hydrogen bonds and serves as proton donor, as well as a hydrogen bond acceptor. Moreover, methacrylic acid interacts ionically with the amine group of templates. Polar functionalities like carboxylic acids, carbamates, carboxylic esters are attracted via hydrogen bonding. Methacrylic acid is suitable for templates containing Brönsted basic or hydrogen bonding functional groups close to the stereogenic center and forms stable cyclic hydrogen bonds with templates containing acid, amide or functionalised nitrogen heterocycles. MAA is broadly applicable but not a universal monomer for the generation of high affinity sites. In comparison to MAA 4-Vinylpyridine (4-VP) provides better selectivity for templates containing acid groups due to its basic functionality. Additionally the p-electron system of the 4-VP benzole ring system interacts with positive charges forming ionic bonding. This property makes 4-VP an interesting candidate for MIPs suitable for applications in aqueous environments. In general, a single sort of functional monomer is used in an imprinting protocol.

Nevertheless, there have been a number of approaches where combinations of two or more functional monomers, giving ter-polymers or higher, have yielded polymers with better recognition abilities than the recognition observed with the corresponding copolymers.

(c) Cross-linking agent: The cross-linking monomer is responsible for mechanical and thermal stability of the polymer. It should be able to sufficiently freeze the pre-polymerisation complex. On the other hand it should release the template after the imprinting process easily and give access to the substrate for rebinding. Hence, template leaking from the polymer is very low and the polymer backbone provides enough micro-, macro- and meso-channels for the substrate to rapidly diffuse to the binding site. The cross-linking polymer is another key-factor for the selectivity due to its structure-stabilizing effect on cavities (<10%). Ethyleneglycol dimethacrylate (EDMA), or trimethylolpropane trimethacrylate (TRIM) have successfully been used.

(d) Porogen/solvent: The solvent plays a very important role in the generation of molecularly imprinted polymers. Three major characteristics regulate the choice of the solvent to be used: (1) it influences the structure of the MIP extensively. Besides the fact that (2) it has to dissolve template, monomer and cross-linker, (3) the solvent controls the porosity, the polymer morphology and governs the strength of non-covalent interactions.

Best solvents for molecular imprinting have a very low dielectric constant like toluene or chloroform (i.e. (at 20°C below 40, preferably below 20, especially below 10). MIPs imprinted with non-polar solvents mostly exhibit the best recognition for an analyte. Non-polar solvents do not disturb the interaction between template and monomer in contrary to water, which is highly polar. Nevertheless, in most cases the choice of solvent is dependent on the solubility of the print molecule
The electrostatic interactions of the pre-polymerisation complexes are sensitive to the presence of polar protic solvents. The degree of dissociation of ion-pairs is strongly dependent on

the solvent. In solvents of high dielectricity (>40), such as water, ion association is only noticeable in solutions at very high concentrations. On the other hand, practically no free ions are found in solvents of dielectric constants less than 10, e.g. toluene and dichloromethane. In solvents of intermediate dielectric constant, e.g. acetonitrile or acetone, the degree of ion association is dependent on additional factors, such as size and charge distribution of the ions. Most molecular imprints in the present invention were generated in acetonitrile (MeCN) and acetone as porogen. Due to its poor hydrogen bonding there is little ability to compete with the hydrogen bonding sites of the template or the polymer binding sites. Moreover, acetonitrile and acetone are polar enough to dissolve a large number of compounds and solvate the methacrylate polymer backbone well. Alternatively, uniform spherical polymer beads can be prepared directly by suspension polymerisation.

Molecularly imprinted microgel particles are prepared from highly diluted monomer solutions. The microgels are composed of intramolecularly crosslinked primary particles formed through multiple crosslinking. When the dilution factor of the monomer solution is increased, the physical form of the imprinted polymers changes from phase-separated inhomogeneous blocks to macrogel particles and finally to discrete microgel particles (Funke et al, 1998).

(e) The initiator: Polymerisation is generally performed at reaction times between 16 and 48 hours, depending on the batch size and format. A number of different photo- and/or thermolabile initiators have been used, the most common being 2,2'-azobis-(2,4-dimethylvaleronitrile) (ABDV) and azobis-(isobutyronitrile) (AIBN). The azobisnitriles are decomposed by heat (ABDV: 40°C; AIBN: 60°C) or UV light, resulting in N_2 and two metastable radicals. The polymerisation process involves three principal phases: initiation, propagation, and termination. The ability of O_2 to accept an additional electron from the radicals leads to premature chain-termination. Hence, the monomer mixture is usually sparged with N_2 or He prior to polymerisation. Moreover, templates with antioxidant properties, such as certain phenolic compounds, or molecules with long con-

jugated p-electron systems, may act as scavengers to inhibit polymerisation and/or potentially be covalently incorporated in the MIP. Electrostatic interactions are anticipated to be mainly responsible for generating the MIP binding sites. Hence, it has been assumed that polymerisation at low temperatures would be beneficial for the imprinting process. It was found that the separation factor (a) increases with decreasing temperature of polymerisation using AIBN at 0°C. On the other hand Sellergren and Shea observed unchanged selectivity by thermal polymerisation. They also showed that by finishing photo initiated polymerisation with heating (120°C, 24h) higher saturation capacities and improved chromatographic performance is obtained (Sellergren et al, 1993).

(f) Ratio of template, functional monomer and cross-linking monomer: Besides the right choice of monomer, cross-linker, solvent and template to obtain an useable MIP of high selectivity, the ratio of "template - functional monomer - crosslinker" is of high importance. A typical ratio for MIP based on a MAA-EDMA copolymer is 1:8:40 and 1:4:4 for MAA-TRIM co-polymers. While experiments have been conducted down to a template-functional monomer ratio of 1:500, an imprinting effect was only seen down to 1:50.

For the present invention with respect to mycotoxin specific polymers, preferred template:monomer ratios are 1:1 to 100, especially 1:1 to 12; preferred template:crosslinker ratios are 1:1 to 100, especially 1:5 to 20. Even more preferred ratios are 1:5 to 20, especially 1:6 to 12, (template:monomer) and 1:20 to 100, especially 1:30 to 80 (template:crosslinker).

Selectivity of the imprinted polymer depends on the orientation of the functional groups inside the cavities and the shape of the cavities. Selectivity increases with the number of binding interactions. First of all, during polymerisation, the interaction between binding site and template needs to be stable. The template should be extracted after polymerisation under mild conditions and as complete as possible (no leaching). For rebinding experiments and practical application the equilibration with substrate molecules has to be rapid and reversible. Non-co-

valent imprinting based on acrylic acid requires a fourfold excess of binding sites to ensure good selectivity. Only 15% of the cavities show re-uptake of a template under these conditions; the remaining 85% are irreversibly lost for use in separation, probably because of shrinking of the cavities. In general, the design depends on the desired application of the matrix either as HPLC stationary phase (rapid adsorption/desorption: monodisperse spherical particles) or solid-phase extraction (large particles: 50mm: bulk polymerisation and subsequent grinding). For the first, the binding site distribution is often characterized by a small number of high affinity high selectivity binding sites and a larger class of less selective, low affinity sites. Possible solution therefore is stabilization of the template assemblies, heat treatment of the materials, and selective blocking of the low affinity non-selective sites. For the latter, the column efficiency is of less importance in contrast to the obtainable recovery, the column load capacity, affinity and selectivity. Whereas MIPs can be prepared in various formats, the most common route is their preparation as bulk polymer with the steps of polymerisation, grinding, sieving, sedimentation and (as described hereinafter as preferred embodiment):

1) Polymerisation: is initiated by irradiation of UV light or heat. A porous polymer monolith is obtained. As the strength of hydrogen bonds and ionic interactions increases with decreasing temperature, initiation by UV irritation should be the first choice for non-covalent imprinting protocols because it can be performed at low temperatures. However, with light-sensitive templates or if solubility problems occur, heat initiation might be preferred.

2) Particle formation: The polymer is mechanically ground to obtain particles with diameters in the µm range. The particles are sieved (usually 25-75µm mesh size) and sedimented repeatedly from acetone to obtain a uniform particle size. In general, yields around 30-50% of the original bulk MIP are obtained. Alternatively, uniform spherical polymer beads can be prepared directly by suspension polymerisation. Moreover, imprinted polymers particles or beads can be rendered magnetic by the inclu-

sion of iron oxide, which enables facile removal of the polymer from the solvent.

3)Extraction: The template and unreacted monomer molecules are extracted from the MIP by repeatedly using polar solvents such as methanol, often in combination with acetic acid. This can be done either in a batch format or in a column depending on the subsequent application. The recovery of template by extraction depends on the size of the template. Although it has been estimated that up to 99% is removed in certain cases, continuous leaching may present problems for application of MIPs in certain cases, such as ultra-trace analysis. These particles are dried under vacuum.

4)Characterisation: The designed MIP has to be tested for its binding capacity of the pre-specified analyte. Usually only 15% of formed cavities can re-bind analyte molecules. Additionally the affinity for the analyte has to be determined (e.g. association constant, dissociation constant) as well as the selectivity for the analyte. There are several ways to determine these parameters. Most common is the characterisation as stationary phase in HPLC. Shift of retention time, peak asymmetry and capacity as well as separation abilities are measured. The application of MIP HPLC columns is also preferred. Molecularly imprinted binding assays using radio-labelled analyte molecules can be more easily applied with all kinds of polymers and various kinds of particle sizes. This test format preferably resembles the format of a conventional ELISA test. Spectrometric detection and immunoassays are rarely used due to sensitivity problems and the lack of availability of antibodies against the analyte, respectively.

Although the delivery of MIPs has shown to be possible for selected organic templates, many challenges in applications for MIPs are given, if a specific organic structure, such as mycotoxins according to the present invention, should be addressed

Although there are very positive and inspiring applications of MIPs presented in the art, the universal application of MIPs in an industrial and commercial way remains still a challenge:

- Imprinting and recognition in aqueous solvents is still a mayor difficulty in the generation of polyacrylic MIPs and more difficult than described in regular review articles: The presence of polar solvents, especially water, can disturb the formation of the pre-polymerization complex during imprinting. The use of polymers in aqueous buffer initially imprinted in non-polar solvents has been demonstrated to be feasible, although sometimes with altered selectivity.

- Binding strength and binding-site heterogeneity are of relevance because they are responsible for the peak tailing in HPLC chromatograms making these polymers less favorable for chromatographic separations and/or solid-phase extraction applications.

- The elution of the template is yet a mayor obstacle. There are still various applications like in solid-phase extraction (SPE) where template bleeding is of major importance leading to increased findings in the determination of analytes in a narrow concentration range.

- So far, few results on the imprinting of larger structures or proteins have been published. Despite of some positive results the imprinting of larger structures is still a major challenge in molecular imprinting.

- Alternative assay formats are needed to replace radioactive substances for competitive molecularly imprinted assays (MIA).

- Last but not least one key factor is sometimes neglected. Even though the material cost are generally very low for the preparation of MIPs they can increase tremendously if the template is expensive.

The present invention provides mycoctoxin MIPs. Therefore mycoctoxins or structural analogs of mycoctoxins are used as templates. Preferred templates are calonectrin, deacetylcalonectrin, 7alpha,8alpha-dihydroxy-calonectrin, 7-hydroxycalonetrin, 8-hydroxy-calonectrin, 3-acetyldeoxy-nevalenol,

15-acetoxydeoxy-nivalenol, 3-acetyl-4,7-deoxynivalen, 3,15-diacetyl-deoxynivalenol, 4,7-dideoxynivalenol, deoxynivalenol, fusarenon-X, nivalenol, diacetylnivalenol, 4-acetyl-scirendiol, diepoxy-diacetyl-scirpenol, 4,15-diacetylscirpendiol, 3,4-diacetylscirpenol, 3,15-diacetylscirpenol, 4,15-diacetylscirpenol, 4,15 -diacetylscirpentriol, 7-hydroxy-isotrichodermin, 8-hydroxy-isotrichodermin, isotrichodermin, 3-monoacetylscirpenol, 15-monoacetylscirpenol, 8-oxodiacetylscirpenol, scirpentriol, triacetylscirpen, 15-acetyl-T-2-tetraol, acetyl T-2 toxin, 3,15-diacetyl-7,8-dihydroxy-12,13-epoxytrichotec-9-en, HT-2, 3'-hydroxy-HT-2, 3-hydroxytrichothecen, 3'-hydroxy-T-2-triol, neosolaniol, neosolaniol-monoacetat, NT-1 toxin, NT-2 toxin, 4-propanoyl HT-2, sporotrichiol, 4,8,15-triacetylscirpendiol, T-2 tetraol, T-2 toxin, 6'8'-dihydroxyzearalen, 5-formylzearalenon, 3'-hydroxyzearalenon, 7'-dehydrozearalenon, 8'-epi-hydroxyzearalenon, 8'-hydroxyzearalenon, zearalanon, zearalanol, zearalenol - cis, a,b,zearalenol - trans, zearalenon-F2, butenolid, FS-1, FS-2, fusarin C, fusariocin C, fusarochromanon, 4-ipomeanol, moniliformin, poaefusarin, sporofusarin, trichotriol, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, aflatoxin M2, 4R-hydroxyochratoxin A, 4S-hydroxyochratoxin A, 10-hydroxyochratoxin A, fumonisin B1, fumonisin B2, fumonisin B3 or mixtures thereof.

Preferred functional monomers are selected from the group comprising 4-vinylpyridine (4-VP), methacrylic acid (MAA), 2-trifluoromethylacryic acid (TFM), methylmethacrylates or other alkylmethacrylates, alkylacrylates, allyl or arylacrylates and methacrylate, cyanoacrylate, styrene, alpha-methylstyrene, vinylester, especially vinylacetate, vinylchloride, methylvinylketon, vinylidenchloride, acrylamide, methacrylamid, acrylonitril, methacrylonitril, 2-acetamidoacrylic acid, 2-(acetoxyacetoxy)ethylmethacrylate 1-acetoxy-1,3-butadiene, 2-acetoxy-3-butenenitrile, 4-acetoxystyrene, acrolein, acroleindiethylacetal, acroleindimethylacetal, acrylamide, 2-acrylamidoglycolic acid, 2-acrylamido-2-methylpropansulfonic acid, acrylic acid, acrylanhydride, acrylonitrile, acryloylchloride, (R)-alpha-acryloxy-beta, beta'-dimethyl-g-butyrolactone, N-acryloxysuccinimide N-acryloxytri.s(hydroxymethyl) aminomethane, N-acrylolychloride, N-acryloylpyrrolidone, N-acryloyl-

tris(hydroxymethyl) amino methane, 2-amino ethyl methacrylate, N-(3-aminopropyl)methacrylamide, (o,m,oder p)-amino-styren,t-amylmethacrylate, 2-(1-aziridinyl)ethylmethacrylate, 2,2'-azobis-(2-amidinopropane), 2,2'-azobisisobutyronitrile, 4,4'-azobis-(4-cyanovaleric acid), 1,1'-azobis-(cyclohexanecarbonitrile);2,2'azobis-(2,4-dimethylvaleronitrile); 4-benzyloxy-3-methoxystyrene;2-bromoacrylic acid; 4-bromo-1-butene; 3-bromo-3,3-difluoropropane; 6-bromo-1-hexene; 3-bromo-2-methacrylonitrile: 2-(bromomethyl)acrylic acid; 8-bromo-1-octene; 5-bromo-1-pentene; cis-1-bromo-1-propene;.beta.-bromostyrene;p-bromostyrene, bromotrifluoro ethylene; (+/-)-3-buten-2-ol;1,3-butadiene;1,3-butadiene-1,4-dicarboxylic acid 3-butenal diethyl acetal; 1-butene; 3-buten-2-ol; 3-butenyl chloroformate; 2-butylacrolein; N-t-butylacrylamide;butyl acrylate; butyl methacrylate; (o,m,p)-bromostyrene; t-butyl acrylate; (R)-carvone; (S)-carvone; (-)-carvyl acetate; cis 3-chloroacrylic acid; 2-chloroacrylonitrile; 2-chloroethyl vinyl ether; 2-chloromethyl-3-trimethylsilyl-1-propene; 3-chloro-1-butene; 3-chloro-2-chloromethyl-1-propene; 3-chloro-2-methyl propene; 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; 3-chloro-1-phenyl-1-propene; m-chlorostyrene; o-chlorostyrene; p-chlorostyrene; 1-cyanovinyl acetate; 1-cyclopropyl-1-(trimethylsiloxy)ethylene; 2,3-dichloro-1-propene; 2,6-dichlorostyrene; 1,3-dichloropropene; 2,4-diethyl-2,6-heptadienal; 1,9-decadiene; 1-decene; 1,2-dibromoethylene; 1,1-dichloro-2,2-difluoroethylene; 1,1-dichloropropene; 2,6-difluorostyrene; dihydrocarveol; (+/-)-dihydrocarvone; (-)-dihydrocarvyl acetate; 3,3-dimethylacrylaldehyde; N,N'-dimethylacrylamide; 3,3-dimethylacrylic acid; 3,3-dimethylacryloyl chloride; 2,3-dimethyl-1-butene; 3,3-dimethyl-1-butene; 2-dimethyl aminoethyl methacrylate; 2,4-dimethyl-2,6-heptadien-1-ol; 2,4-dimethyl-2,6-heptadienal; 2,5-dimethyl-1,5-hexadiene; 2,4-dimethyl-1,3-pentadiene; 2,2-dimethyl-4-pentenal; 2,4-dimethylstyrene; 2,5-dimethylstyrene; 3,4-dimethylstyrene; divinyl benzene; divinyltetramethyl disiloxane; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-porphine; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-propionic acid; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-propionic acid disodium salt; 3,9-divinyl-2,4,8,10-tetraoraspiro[5,5]undecane; divinyl tin dichloride; 1-dodecene; 3,4-epoxy-1-butene; 2-ethyl acrolein; ethyl acrylate; 2-ethyl-1-

butene; (+/-)-2-ethylhexyl acrylate; (+/-)-2-ethylhexyl methacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol triacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate; ethyl methacrylate; ethyl vinyl ether; ethyl vinyl ketone; ethyl vinyl sulfone; (1-ethylvinyl)tributyl tin; m-fluorostyrene; o-fluorostyrene; p-fluorostyrene; glycol metacrylate (hydroxyethyl methacrylate); GA GMA; 1,6-heptadiene; 1,6-heptadienoic acid; 1,6-heptadien-4-ol; 1-heptene; 1-hexane-3-ol; 1-hexene; hexafluoropropene; 1,6-hexanediol diacrylate; 1-hexadecene; 1,5-hexadien-3,4-diol; 1,4-hexadiene; 1,5-hexadien-3-ol; 1,3,5-hexatriene; 5-hexen-1,2-diol; 5-hexen-1-ol; hydroxypropyl acrylate; 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene; isoamyl methacrylate; isobutyl methacrylate; isoprene; 2-isopropenylaniline; isopropenyl chloroformate; 4,4'-isopropylidene dimethacrylate; 3-isopropyl-alpha-alpha-dimethylbenzene isocyanate; isopulegol; itaconic acid; itaconalyl chloride; lead(II)acrylate; (+/-)-linalool; linalyl acetate; p-mentha-1,8-diene; p-mentha-6,8-diene-2-ol; methyleneamino acetonitrile; methacrolein; [3-(methacryloylamino)-propyl]trimethylammonium chloride; methacrylamide; methacrylic acid; methacrylic anhydride; methacrylonitrile; methacryloyl chloride; 2-(methacryloyloxy)ethyl acetoacetate; (3-methacryloxypropyl)trimethoxy silane; 2-(methacryloxy)ethyl trimethyl ammonium methylsulfate; 2-methoxy propene (isopropenyl methyl ether; methyl-2-(bromomethyl)acrylate; 5-methyl-5-hexen-2-one; methyl methacrylate; N,N'-methylene bisacrylamide; 2-methylene glutaronitrile; 2-methylene-1,3-propanediol; 3-methyl-1,2,-butadiene; 2-methyl-1-butene; 3-methyl-1-butene; 3-methyl-1-buten-1-ol; 2-methyl-1-buten-3-yne; 2-methyl-1,5-heptadiene; 2-methyl-1-heptene; 2-methyl-1-hexene; 3-methyl-1,3-pentadiene; 2-methyl-1,4-pentadiene; (+/-)-3-methyl-1-pentene; (+/-)-4-methyl-1-pentene; (+/-)-3-methyl-1-penten-3-ol; 2-methyl-1-pentene; .alpha.-methyl styrene; t-alpha-methylstyrene; t-beta.-methylstyrene; 3-methylstyrene; methyl vinyl ether; methyl vinyl ketone; methyl-2-vinyloxirane; 4-methylstyrene; methyl vinyl sulfone; 4-methyl-15-vinylthiazole; myrcene; t.beta.-nitrostyrene; 3-nitrostyrene; 1-nonadecene; 1,8-nonadiene; 1-octadecene; 1,7-octadiene; 7-octene-1,2-diol; 1-octene; 1-octen-3-ol; 1-pentadecene; 1-pentene; 1-penten-3-ol; t-2,4-pentenoic acid; 1,3-pentadiene; 1,4-pentadiene; 1,4-pentadien-3-ol; 4-pen-

ten-1-ol; 4-penten-2-ol; 4-phenyl-1-butene; phenyl vinyl sulfide; phenyl vinyl sulfonate; 2-propene-1-sulfonic acid sodium salt; phenyl vinyl sulfoxide; 1-phenyl-1-(trimethylsiloxy)ethylene; propene; salfrole; styrene (vinyl benzene); 4-styrene sulfonic acid sodium salt; styrene sulfonyl chloride; 3-sulfopropyl acrylate potassium salt; 3-sulfopropyl methacrylate sodium salt; tetrachloroethylene; tetracyano ethylene; tetramethyldivinyl siloxane; trans 3-chloroacrylic acid; 2-trifluoromethyl propene; 2-(trifluoromethyl)propenoic acid; 2,4,4'-trimethyl-1-pentene; 3,5-bis(trifluoromethyl)styrene; 2,3-bis(trimethylsiloxy)-1,3-butadiene; 1-undecene; vinyl acetate; vinyl acetic acid; 4-vinyl anisole; 9-vinyl anthracene; vinyl behenate; vinyl benzoate; vinyl benzyl acetate; vinyl benzyl alcohol; 3-vinyl benzyl chloride; 3-(vinyl benzyl)-2-chloroethyl sulfone; 4-(vinyl benzyl)-2-chloroethyl sulfone; N-(p-vinyl benzyl)-N,N'-dimethyl amine; 4-vinyl biphenyl (4-phenyl styrene); vinyl bromide; 2-vinyl butane; vinyl butyl ether; 9-vinyl carbazole; vinyl carbinol; vinyl cetyl ether; vinyl chloroacetate; vinyl chloroformate; vinyl crotanoate; vinyl cyclohexane; 4-vinyl-1-cyclohexene; 4-vinylcyclohexene dioxide; vinyl cyclopentene; vinyl dimethylchlorosilane; vinyl dimethylethoxysilane; vinyl diphenylphosphine; vinyl 2-ethyl hexanoate; vinyl 2-ethylhexyl ether; vinyl ether ketone; vinyl ethylene; vinyl ethylene iron tricarbonyl; vinyl ferrocene; vinyl formate; vinyl hexadecyl ether; vinylidene fluoride; 1-vinyl imidizole; vinyl iodide; vinyl laurate; vinyl magnesium bromide;vinyl mesitylene; vinyl 2-methoxy ethyl ether; vinyl methyl dichlorosilane; vinyl methyl ether; vinyl methyl ketone; 2-vinyl naphthalene; 5-vinyl-2-norbornene; vinyl pelargonate; vinyl phenyl acetate; vinyl phosphonic acid, bis(2-chloroethyl)ester; vinyl propionate; 4-vinyl pyridine; 2-vinyl pyridine; 1-vinyl-2-pyrrolidinone; 2-vinyl quinoline; 1-vinyl silatrane; vinyl sulfone; vinyl sulfone (divinylsulfone); vinyl sulfonic acid sodium salt; o-vinyl toluene; p-vinyl toluene; vinyl triacetoxysilane; vinyl tributyl tin; vinyl trichloride; vinyl trichlorosilane; vinyl trichlorosilane (trichlorovinylsilane); vinyl triethoxysilane; vinyl triethylsilane; vinyl trifluoroacetate; vinyl trimethoxy silane; vinyl trimethyl nonylether; vinyl trimethyl silane; vinyl triphenyphosphonium bromide (triphenyl vinyl phosphonium bromide); vinyl tris-(2-methoxyethoxy)silane; vinyl 2-

valerate; acrylate-terminated or otherwise unsaturated urethanes, carbonates, and epoxides, especially allyl diglycol carbonate, glycidyl acrylate, glycidyl methacrylate, allyl glycidyl ether, and 1,2-epoxy-3-allyl propane; or mixtures thereof.

For providing mycotoxin MIPs, the porogenic solvent is preferably selected from the group comprising acetonitrile, methanol, acetone and other apolar solvents, benzene, toluene, chloroform, dichlormethane, tetrahydrofurane, dimethylformamide, dimethylsulfoxide, ethanol, 1-propanole, methanol, water or mixtures thereof.

In the present invention, preferred crosslinkers are selected from the group comprising di-, tri- and tetrafunctional acrylates or methacrylates, divinylbenzene (DVB), alkyleneglykols, polyalkyleneglycoldiacrylates und methacrylates, especially ethylenglycoldimethacrylate (EDMA), trimethyltrimeth-acrylate (TRIM), or ethylenglycoldiacrylate, vinyl or allylacrylates or methacrylates, diallyldiglycoldicarbonate, diallylmaleate, diallylfumarat, diallylitaconate, vinylesters, especially divinyloxalate, divinylmalonate, diallylsuccinate, triallylisocyanurate, dimethacrylates or diacrylates of bis-ophenol A or ethoxylated bis-phenol A, methylene or polymethylene bisacrylamide or bismethayrylamide, hexamethylenebisacrylamide or hexamethylenbismethacrylamide, di(alkene)tertiary amine, trimethylolpropantriacrylate, pentaerythritoltetraacrylate, divinylether, divinylsulfone, diallylphtalates, triallylmelamine, 2-isocyanatoethylmethacrylate, 2-isocyanatoethylacrylate, 3-isocyanatopropylacrylate, 1-methyl-2-isocyanatoethylmethacrylate, 1,1-dimethyl-2-isocyanaotoethylacrylate, tetraethyleneglycoldiacrylate, tetraethyleneglycoldimethacrylate, hexanedioldimethacrylate, hexanedioldiacrylate or mixtures thereof.

In the method according to the present invention, any suitable polymerisation starters may be used, however, preferably 2,2'-azobis-isobutyronitrile (AIBN), 2,2'-azobis-(2,4-dimethylvaleronitrile) (ABDV) or mixtures thereof are used as starters for said co-polymerisation.

The co-polymerisation is preferably carried out in an oxygen-free atmosphere.

According to a preferred embodiment, the co-polymerisation is started by photoinitiation or by thermal initiation.

According to another aspect of the present invention, mycotoxin imprinted polymers are provided. It was surprising that with the methods according to the present invention polymers could be provided which exhibit a mycotoxin dRT (retention time difference) value of more than 0,7 thereby showing a satisfactory and specific retention of mycotoxins. Specifically, these polymers are obtainable by a method according to the present invention. With such methods, even mycotoxin dRT values of 1,0 or more, especially of 3,0 or more, are achieved. This was clearly not focusable in the prior art which pointed into the opposite direction (see e.g. Jodlbauer et al. 2002), specifically for molecules having the chemical structure of DON and 20N or similar chemical structures.

The preferred specific binding molecules of the polymers according to the present invention are nivalenole, deoxynivalenole, zearalenone, T2 toxin, HT2 toxin, or mixtures thereof, since these are the commercially important ones. The examples of the present invention have also specifically designed in view of these preferred mycotoxins, yet without being restricted thereto. In the examples, the specifically preferred functional monomers, 4-vinylpyridine, methacrylic acid, 2-trifluoromethacrylic acid or mixtures thereof, have been used for building up the polymers.

Specifically preferred mycotoxins to be imprinted according to the present invention include: (1) trichothecene A und B (12,13-Epoxytrichothec-9-en ring system)M especially HT-2 toxin, Diacetoxyscirpenol, T-2 Toxin, T-2 Ttetraol, Nivalenol, Deoxynivalenol and Fusarenon-X and (2) all Zearalenone metabolites: Zearalenon, Zeranol, alpha-Zearalanol, Taleranol, beta-Zearalanol, alpha-Zearalenol, beta-Zearalenol.

Other aspects of the present invention relate to specific uses

of the mycotoxin binding method or the mycotoxin imprinted polymers according to the present invention.

For example, the mycotoxin imprinted polymers are preferably used for solid phase extraction of mycoctoxins from mycotoxin containing solutions or suspensions, as food additives, for the preparation of a medicament for treating or preventing mycotoxin-caused disorders, for diagnosing the presence of mycotoxins in a tissue sample or fluid sample from humans, from animals or from plants.

According to another preferred aspect, the present polymers may be used in or as biosensors for detecting the presence and/or the amount of mycotoxins in a sample, especially a biological, environmental or industrial sample.

A preferred use on industrial scale is cleansing of mycotoxin contaminated solutions, suspensions or aerosols.

Food and beverage analysis and decontamination are preferred fields of use as well as water quality analysis and decontamination, e.g. if poisoned with mycotoxins (homeland security and public security application).

All these preferred uses, but also other uses are positively affected by the robust design and nature of the present polymers. There is no mandatory need for careful laboratory treatment of these substances, which makes them extremely suitable for field testing or, generally, using them without being dependent on certain facilities, such as laboratories.

The invention is further described in the following examples and the figures, yet without being limited thereto.

Figures:

Figure 1. Schematic of non-covalent imprinting. (1) self assembly of the print molecule (DON), functional monomers and cross-linking monomers. After formation of the pre-polymerisation complex (2) the cross-linking monomer polymerises, (3)

after extraction of the print molecule, (4) the binding site is free for specific uptake of the substrate.

Figure 2. DON and ZON: possible OH bonding with bond strength estimates. (ww) very weak, (w) weak, (s) strong, (ss) very strong. Quercetin as 'dummy template' has similarity to ZON in structure and functional groups at the aromatic ring.

Figure 3. Chromatograms of acetone, 3-acetyl DON, fusarenon-X and DON in (a) the anti-DON MIP column DON-MAA and (b) the blank (control) polymer DON-MAA-BP. Stationary phase: MAA, EDMA; mobile phase: acetonitrile, flow rate 0.5 ml/min at ~8 bar back-pressure, stainless steel HPLC columns (250 x 4.6 mm id); DON concentration was 1 µg/ml each. Acetone concentration was 10 µl/ml each.

Figure 4. Chromatograms of acetone and ZON on (a) ZON-4VP-BP, (b) ZON-4VP: anti-ZON imprinted polymer (stainless steel HPLC column: 150 x 4.6 mm id), flow rate 0.5 mL/min, mobile phase MeCN, and finally (c) QUE-4VP-BP and (d) QUE-4VP: anti-quercetin imprinted polymer (stainless steel HPLC column: 250 x 4.6 mm id), flow rate 1 ml/min, mobile phase MeCN with 0.1 % HAc. ZON concentration was 0.1 µg/ml each, acetone concentration was 10 µl/ml each.

E x a m p l e s :

1.Applications of Mycotoxin Molecularly Imprinted Polymers

Mycotoxin MIPs have numerous applications, especially in similar areas of application as conventional antibodies such as Food Analyses (Ramström et al., 2001). Due to their higher mechanical and thermal stability, MIPs can be used in applications working in harsh environments. Preferred applications of MIPs are for separation procedures in HPLC columns and solid-phase extraction (SPE), as recognition matrix in chemical and biosensors, in molecularly imprinted sorbent assays (MIA) similar to a radio-immuno assays (RIA) and as enzyme mimics.

1.1. Characterization and Application as Stationary HPLC Phase

Liquid chromatography has been developed into a standard technique for liquid separation and is routinely used to separate and measure a wide range of analytes. A rough summary of conventionally applied stationary phases for HPLC is given in Waters Corporation (1999). The use of MIPs as stationary phases for LC is by far the most extensively studied application of imprinted polymers and has been reviewed in some excellent papers (Kempe et al, 1995; Remcho et al, 1999). After wet sieving of the MIP and filling an HPLC column under high-pressure stationary phases are obtained with selectivity for a predetermined substrate, or a class of compounds. Especially the separation of enantiomers has been addressed by the generation of chiral stationary phases based on MIPs. Besides, MIP stationary phases are used for the characterisation of MIPs. Retention time is recorded for the imprinted analyte. The retention time is compared to structural analogues (ideally a stereoisomer) and should be higher then the used reference. The higher the difference the more binding cavities specific for the imprinted template have been formed during the imprinting procedure. The recognition properties of the polymers are then assessed by comparing the retention times (tR) or capacity factors (k') of the template with the structurally related analogues. Following parameters are generally used for the characterisation of MIPs:

(a) Partition coefficient K: describes the ratio of analyte distribution in the mobile phase and the stationary phase. The partition coefficient K cannot be directly deduced from the chromatogram.

$$K = cS/cM$$

cS ... concentration in the stationary phase
cM... concentration in the mobile phase

(b) Total retention time tR: time required for the analytes in the mobile phase to pass through the column (at the peak maximum). Under defined chromatographic conditions tR is a characteristic signature of the analyte, but is influenced by (i) sample load and (ii) mobile phase.

(i) The sample load is a frequent problem of enantiomer separation: decrease of sample loads leads to an increase in both, retention and selectivity. Furthermore the mass trans fer kinetics are particularly slow in organic mobile phases. This problem is addressed by decreasing the flow rate.
(ii) When increasing the aqueous content in the mobile phases polar templates usually become less retained on MIPs, whereas templates of moderate to low polarity become more retained. The latter increase in retention is due to the hydrophobic effect. Thus, in contrast to other types of affinity phases with biological recognition elements (immunoaffinity phases), imprinted phases behave more like reversed phases when the aqueous content is high. This leads to pronounced specific binding, frequently in the form of total retention of all hydrophobic compounds, and can be reduced by addition of an organic modifier or a detergent.

(c) Hold-up time tM: time required for an analyte in the mobile phase or mobile phase molecules who do not interact with the stationary phase (not retained; also called void marker) to pass through the column (dead time).

(d) Capacity factor k´: as quotient of retention time tR and hold-up time tM the capacity factor describes the enhancement of separation by the selective solid phase.

$$k' = K \cdot VS/VM = K/b = (tR-tM)/tM = tR'/tM$$

VS, VM... volume of the stationary or the mobile phase
b ... phase ratio
tR´... adjusted retention time

(e) Separation factor alpha: For two analytes A and B to describe the selectivity of the phase system (A being the print molecule and B the respective test substance):

$$\alpha = \frac{K_B}{K_A} = \frac{k_B'}{k_A'} = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} = \frac{(t_R)_B - t_M}{t_M} \cdot \frac{t_M}{(t_R)_A - t_M}$$

Preferred techniques are reversed phase, normal phase, ion ex-

change, ion suppression and hydrophilic interaction chromatography (for details: see Waters Corporation, 1999)

1.2. Molecularly Imprinted Sorbent Assay (MIA)

The present MIPs are specifically suited as non-biological alternatives to antibodies in competitive radiolabelled molecularly imprinted sorbent assays (MIA). In general 3H or 14C labelled analyte is used as competitive ligand. A molecularly imprinted sorbent assay (MIA) is performed similar to a RIA. The bound radiolabelled analyte is detected via a radioactivity detector. Binding capacity, selectivity, and affinity can be determined by assays using analogous analytes (see: US patent 6 255 461). Selectivity in the micromolar range and lowest KD in the low nanomolar range can be observed. Heterogeneity of binding sites can be calculated by saturation studies.

The assay may also be designed as a competitive fluorescence assay performed with the analyte and an non related fluorescent probe and as an enzyme-linked molecularly imprinted sorbent assay using enzyme labeled substrate as competitive agent in a MIA instead of radio-labeled analytes.

1.3. Molecularly Imprinted Solid-Phase Extraction (MISPE)

The application of molecular imprinting in solid-phase extraction (SPE) is closest to practical realisation and commercial exploration. SPE in its various forms - online or offline - using various types of sorbents have gained tremendous importance in the last decade due to their cheapness and effectiveness. There is an increased need for efficient large scale screening methods in environmental monitoring, food safety surveillance and in clinical applications, which has to be at low-cost, rapid, using automated methods of analysis and having low limits of detection for contaminants, food additives, drugs, narcotics and their metabolites. Solid-phase extraction using MIPs was most recently reviewed by A. Martin-Esteban, 2001.

SPE consists of percolating a known sample volume of a liquid sample through a solid sorbent (in the format of a cartridge,

column or disk) under carefully selected conditions favouring the preferential absorption of the analyte over the matrix components. The analyte of interest is then recovered from the sorbent by elution in a small volume (smaller than the applied sample volume) of an appropriate solvent mixture. High enrichment as well as efficient sample clean up can thus be obtained in one-step. Multi-purpose SPE matrices can be used for hydrophobic analytes. The attainable enrichment and clean up depends on:

- Selectivity
- Affinity of the sorbent for the selected target analyte or analytes
- Sample load capacity for the analytes
- Rate of mass transfer to and from the binding sites
- Minimum desorption volume and thus the enrichment
- Reproducibility of the recovery yields
- Stability and reusability of the sorbent.

Common phases are hydrophobised silica (C8, C18), styrene-divinylbenzene copolymers (PS-DVB) and graphitised carbon black (GCB). Reversed phase materials are characterized mainly by a high sample load capacity and a wide range of trapped analytes. Disadvantages are their poor selectivity, their narrow pH stability range and limited breakthrough volumes for hydrophilic analytes. In the field of mycotoxin SPE, specificity has heretofore only be accepted to be provided by the development of high affinity SPE matrices based on biological recognition elements like enzymes, antibodies or cells.

Nevertheless they have several drawbacks, including minor robustness and reproducibility, demanding receptor immobilization and time and cost involved in providing the receptor. Classical solid-phase extraction methods lack two features where MISPE is of advantage: specificity and cheapness. The most versatile approach in gaining MISPE matrices is based on the self-assembly approach. MIPs may preferably used in cartridge format and in offline mode. Aqueous samples can be directly applied to the column (non-selective extraction of hydrophobic analytes from aqueous media, selective washing procedures remove non-selectively bound matrix components) or have to be transferred to organic solvents of lower polarity (adsorption occurs similarly to

the LC normal phase operation). In MISPE, the washing and elution conditions need to be carefully optimised in terms of ionic strength, pH, and solvent composition. Best results are usually obtained when using the porogenic solvent as wash solvent. Possible disadvantages can be compatibility problems (many of the solvents are not miscible with water) and the drying of MIP cartridges prior to the washing step. Elution of more weakly bound analytes is generally performed by methanol or water. For more strongly bound analytes the same solvents with the addition of small amounts of acids (acetic acid, trifluoroacetic acid) or bases (TEA) are used.

1.4. Molecularly Imprinted Films for Biomimetic Sensors

The application of molecularly imprinted polymers (MIPs) in sensor technology has been reviewed recently (Haupt et al, 2000; Al-Kindy et al, 2000). The combination of specific molecular recognition capabilities with stability and robustness is especially attractive to applications in the sensor field (WO 01/77667 A2). Sensing layers based on MIPs are physically and chemically more stable than conventional recognition elements (e.g., antibodies, proteins, or cells) when stored or used. They can be used in aqueous solvents as well as organic solvents and tailored specificity guarantees the availability of all kinds of organic molecules for imprinting in the same quality throughout all production batches. Methacrylate-based imprinted polymers have the affinity and selectivity required to act as recognition elements in a sensing device. In biosensors, a signal is generated upon binding of the analyte to the recognition element. The transducer then translates this signal into a quantifiable (one or more physicochemical parameters) output signal. The same general principle applies when MIPs are used as the recognition element instead of a biomolecule.

There are different transduction mechanisms to translate the binding event into a measurable signal:

- Electrochemical sensors, classified as amperometric, potentiometric, conductometric, or field effect transistor-based.
- Mass-sensitive acoustic transducers e.g., surface-acoustic wave

oscillator (SAW), quartz crystal microbalance (QCM).
- Optical sensors e.g., ellipsometry, surface plasmon resonance (SPR) or infrared evanescent field.
- Diffusion through selective membranes: membranes (MIPS) as components of electrochemical sensors (eg. conductometric detection).
- Fluorescence sensor based on the fluorescence of the MIP after analyte binding.

In all cases an important aspect in the design of a MIP-based sensor is to find an appropriate way of interfacing the polymer with the transducer, since the MIP layer has to be in intimate contact with the transducer surface. Thereby, the polymer can either be synthesized in situ at the transducer surface or the surface can be coated with the preformed polymer. Preferred techniques are: electro-polymerisation on conducting surfaces, spin coating and spray coating, sandwich technique, entrapping of nanometer- or micrometer-sized particles into gels or behind a membrane, spin coating of a suspension of MIP-particles in an inert, soluble polymer.

Coating of ATR crystals by the sandwich technique:
Methacrylate based polymers are characterized by their rigidity, which is a necessitity for HPLC solid phases, but a dissadvantage for the formation of thin coatings. Due to the change in volume caused by solvent changes and drying, polyacrylic molecularly imprinted polymers usually crack easily on germanium (Ge) or zincselenide (ZnSe) ATR-crystals. Although thin polymer layers can be produced by the sandwich technique with simple laboratory equipment, it has certain limitations. A crucial step in the sandwhich technique is the assembly of ATR crystal, the spacer (inbeneath the polymerisation liquid) and the cover glass.

The spacer defines the thickness of the generated polymer layer and the cover glass is necessary for the generation of an even surface and the exclusion of oxygen, which would prevent polymerization. If the polymerization is initiated by heat, the glass cover also prevents evaporation of the porogen. Otherwise the polymerization will fail, even if oxygen is excluded by

polymerizing under nitrogen gas stream. Three major parameters influence the adheasion of MIPs to the ATR crystal: (i) the choice and amount of solvent, (ii) the ratio between functional monomer and cross-linking monomer, and (iii) the surface properties of the ATR crystal.

(i) Choice and amount of porogen: Using the porogen methanol/water an even and good adhering MIP layer is obtained. But for most imprinting purposes the usage of acetonitrile as porogen is crucial due to hydrogen bonding based interactions between functional monomer and template substrate. Due to different physical parameters of acetonitrile (wetability, rigidity of the resulting polymer) polymers generated with acetonitrile do not adhere to ATR crystals (ZnSe, Ge).

(ii) Ratio between functional and cross-linking monomer MAA: The ratio between MAA and cross-linker can increase the "softness" and therefore the coating properties of the polymer. The stability of the ATR coating is increased e.g. by changing the ratio of MAA:TRIM from 1:1 to 2:1). In addition to the increased softness by the increase of methacrylic acid, the plasticizer dibutylphtalate was applied. As externary plasticizer the polar groups of dibutylphtalate are interacting with the polar groups of the polymer. Hence, the polymer chains are loosened up and the movement of the polymer chains is facilitated. Softness and stretching of the generated polymer increases.

(iii) Surface properties of the ATR crystal: Common ATR-crystal materials are zincselenide or germanium, both materials have been tested as substrate for the formation of MIP layers. Adhesion of poly-acrylate based MIPs to zincselenide was more feasible, because of the high metallic surface of germanium crystals. By optimization of porogen and monomer ratio the generation of even MIP layers was realized.

1.5. Further Applications of MIPs

Screening of chemical or biological libraries has become increasingly important as tool in drug discovery. Combinatorial chemistry will highly contribute to the pursuit of novel lead compounds in the pharmaceutical industry. MIPs, as artificial receptors, could also be employed for the screening of libraries for potential new drugs and enzyme inhibitors. The target com-

pounds (potential mycotoxin mimics) can be specifically recognized by the respective MIPs from an entire library.

2. Molecularly Imprinted Polymers for Deoxynivalenol and Zearalenone

2.1. MATERIALS AND METHODS

Chemicals. Nivalenol, fusarenon-X, 15-acetyl deoxynivalenol, 3-acetyl deoxynivalenol, quercetin, and zearalenone were supplied by Sigma-Aldrich (St. Louis, MO). Gradient grade acetonitrile, methanol and acetone were of HPLC quality and supplied by Merck (Darmstadt, Germany), ethylenglycoldimethacrylat (EDMA), trimethyltrimethacrylate (TRIM), divinylbenzene (DVB), 4-vinylpyridine (4-VP), methacrylic acid (MAA), 2-trifluoromethylacrylic acid (TFM) and 2,2'-azobis-isobutyronitrile (AIBN) were from Sigma-Aldrich. Crystalline DON was provided by BioPure (Tulln, Austria). According to Altpeter and Posselt (Altpeter and Posselt 1994) Fusarium graminearum was inoculated on rice. The isolation of DON included an extraction step, followed by flash chromatography over silica gel and crystallisation of DON from ethyl acetate.

Preparation of the imprinted polymer. The molecularly imprinted polymer (IMP) was prepared by bulk polymerisation. The molar ratios of the ingredients are listed in Table 1 (DON) and Table 2 (ZON). The respective template was dissolved in the porogen in a glass tube. The functional monomer (4-vinylpyridine, methacrylic acid or trifluoromethacrylic acid), the crosslinking monomer (EDMA, TRIM or DVB) and the initiator AIBN (2 mol% of the reactive double bonds) were then added to the solution. The solution was cooled in an ice bath and purged with nitrogen for 5 min. The degassed pre-polymerisation mixture was subsequently polymerised first by photo-initiation at 366 nm (11°C) overnight and at 65°C for six hours. The same procedure without adding a template was used, in order to prepare a non-imprinted polymer (BP) for control experiments.

The obtained polymer was crushed, then ground for 3 min in a mechanical mortar (Retsch, Haan, Germany) and wet-sieved in acetone until particles with a diameter < 25 µm were obtained. The polymer was then sedimented, in order to eliminate particles < 5 µm and for obtaining a uniform particle size. The sedimentation was done in ~250 mL acetone; after two hours the supernatant solution was discarded and fresh acetone was added to the precipitated MIP particles. The majority of small particles was eliminated after five to six sedimentations. Finally, the polymer particles were filtrated, washed with methanol and dried at 45°C for 24 hours.

Preparation of the column. The sieved polymer particles were sonicated in acetone and were packed into stainless-steel HPLC columns (250 or 150 x 4.6 mm if not stated otherwise in the context) using acetone at 200 bar by an air driven fluid pump (Alltech, USA). In order to extract the template molecule from the polymer material, the column was washed on-line with methanol/acetic acid (HAc) (7:1, v/v) at 1 mL min^{-1} until a stable baseline was reached.

HPLC analysis. The HPLC-analysis was performed using a Dionex HPLC (Dionex, Sunnyvale, CA, USA) with a P580 low pressure mixing pump and an UVD-340S diode array detector with a spectral range from 200 - 600 nm. After complete extraction of the template, each column was equilibrated with the mobile phase (MeCN with HAc). The elution was performed at ambient temperature and monitored spectro-photometrically at 200 - 450 nm. The flow rate was kept constant at 0.5 - 1 mL min^{-1} throughout the whole study.

For each chromatographic run about 2 µg of the compounds in 20 µl of the mobile phase were injected, using 0.2 µl acetone as void marker. Capacity factors were calculated as $k'=(t-t0)/t0$ where t is the retention time for the compound and t0 corresponds to the retention time for the void marker. Separation factors were calculated as $a=k'PM/k'TS$, with PM indicating the print molecule and TS the respective test substance. The retention index (RI) was calculated as RI=aCP/aMIP, where MIP and CP indicate the molecularly imprinted polymer and control polymer, respectively.

2.2. RESULTS AND DISCUSSION

The aim of this example was the development and characterisation of molecularly imprinted polymers for the mycotoxins DON and ZON. The major challenge for this study were the high costs for the targeted template substances. Hence, only a few molecularly imprinted test polymers have been prepared after careful consideration of the imprinting procedure. Furthermore, instead of obtaining DON from commercial sources, the isolation of the toxin from fungi cultures was pursued.

Both templates, DON and ZON, have OH groups as part of their molecular structure (see Figure 2). DON has three different hydroxyl groups in 3, 7, and 15 position of the ring system. Whereas the OH-group in position 7 is non-reactive, the other two OH-groups are reactive and are used for coupling spacers to the DON molecule. The OH at position 15 is more reactive than at position 3. This reactivity is the reason for the occurrence of 3-acetyl DON and 15-acetyl DON in nature. It is therefore supposed that this two OH-groups being the most probable non-covalent interaction point for complementary functional monomers, such as methyl-methacrylate or 4-vinylpyridine.

The structure of ZON shows two reactive OH-groups at the main aromatic ring. The two carboxyl-groups at the 14 ring are less attractive for hydrogen bonds due to their minor ability forming such bonds and, secondly, because of their less favourable accessibility due to steric hindering and due to the high flexibility of the large 14-ring system.

Therefore, functional monomers like MAA and 4-VP have been applied, which facilitate hydrogen bonding. The latter is additionally capable of ionic interaction and was successfully used during the generation of anti-quercetin molecularly imprinted solid-phase extraction materials. Acetonitrile has been used

Table 1 List of generated anti-DON molecularly imprinted polymers

type	template [mmol]	functional monomer	cross linker	porogen	molar ratio	dRT 15 cm[1)]	AIBN [mg]
ON-DEAMA	DON, 0.67	DEAMA: 1.34	EDMA: 19 mmol	MeCN: 3.5 ml	1:2:28	0.7	70.1mg
ON-DEAMA-P	-	DEAMA: 1.34	EDMA: 19 mmol	MeCN: 3.5ml	1:14		70.1mg
ON-DVB	DON, 0.50	4-VP: 4mmol	DVB: 20 mmol	MeCN: 4.1ml	1:8:40	0.8	78.8mg
ON-DVB-BP	-	4-VP: 4mmol	DVB: 20 mmol	MeCN: 4.1ml	1:5		78.8mg
ON-MAA	DON, 0.70	MAA: 8.5 mmol	EDMA: 42.3mmol	MeCN: 12ml	1:12:60	3.0[2)]	182mg
ON-MAA-BP	-	MAA: 8.5 mmol	EDMA: 42.3mmol	MeCN: 12ml	1:5		182mg
ON-4VP	DON, 0.68	4-VP: 5.4 mmol	EDMA: 27 mmol	MeCN: 9ml	1:8:40	3.1[2)]	119mg
ON-4VP-BP	-	4-VP: 5.4 mmol	EDMA: 27 mmol	MeCN: 9ml	1:5		119mg

Flow rate is in all cases 0.5ml/min and MeCN as mobile phase.

The column length for DON-4VP and DON-MAA was 25x4.6mm.

previously as porogenic solvent during imprinting procedures, generating reasonably good recognition conditions promoting the assembly between the target molecules and the functional monomers. The hydrogen bonding capacity of acetonitrile is quite poor and, therefore, will not interfere with the relation template-monomer. At the same time acetonitrile is one of the few solvents being able to dissolve the required amount of DON and ZON for the imprinting protocol, mainly because of its high polarity. Solubility of ZON in water is only 20 mg/L; it is slightly soluble in toluol (< 1 mg/mL) and progressively more soluble in acetonitrile (> 27 mg/mL), methylenchloride (> 30 mg/mL), methanol, acetone and in aqueous alkali (see also Hidy et al. 1977). Solubility of DON is even more difficult to handle; only acetonitrile (approx. 20 mg/ml) was found to be suitable (dichlormethane: 7 mg/ml; toluol: 0.4 mg/ml). Table 1 and Table 2 summarize the generated polymers for DON and ZON.

Table 2 List of generated anti-ZON molecularly imprinted polymers

type	template [mmol]	functional monomer	cross-linker	porogen	molar ratio	dRT 15 cm	AIBN
ON-TFM	ZON, 0.16	TFM: 1.26 mmol	EDMA: 6.28 mmol	MeCN: 2.5 ml	1:8:40	0.7	24.8 mg
ON-TFM-BP	-	TFM: 1.26 mmol	EDMA: 6.28 mmol	MeCN: 2.5 ml	1:5		24.8 mg
ON-4VP	ZON, 0.16	4-VP: 1.6 mmol	EDMA: 8 mmol	MeCN: 3 ml	1:10:50	0.9	35 mg
ON-4VP-BP	-	4-VP: 1.6 mmol	EDMA: 8 mmol	MeCN: 3 ml	1:5		35 mg
UE-4VP	Querc, 1.00	4-VP: 8 mmol	EDMA: 40 mmol	Aceton: 15 ml	1:8:40	3.4[1)]	175 mg
UE-4VP-BP	-	4-VP: 8 mmol	EDMA: 40 mmol	Aceton: 15 ml	1:5		175 mg

Instead of 15x4.6mm ID and MeCN as mobile phase, the column length for QUE-4VP was 25x4.6mm and the mobile phase was 0.1% Ac in MeCN, flow rate was in all cases 1.0 ml/min.

HPLC results for DON.

The performance of the prepared MIPs has been assessed via HPLC measurements, investigating the separation behaviour in comparison to very close structural analogues. A separation of a-b isomers, as has been shown previously for confirmation of an imprinting effect (Kempe et al. 1993, Nomura et al. 1998) was not possible due to the lack of commercially available DON isomers. Instead, very similar analogues (in terms of Kow values, structural properties and functional groups) have been selected. Using DVB as cross-linking monomer (DON-DVB and DON-DVB-BP) the achieved change in RT is relatively small. The separation abilities of the molecularly imprinted polymer were not significantly increased. The acetone peak remained unchanged in MIP and control polymer (2.1 in the MIP, 2.14 in the BP), while the DON peak shifts from 5.4 min to 5.9 min in the imprinted polymer. In fact, the DON peak appears more tailed, which is accounted for an imprinting effect due to the generation of binding sites with a heterogeneous affinity distribution. Nivalenol and fusarenon-X show changed RT in the MIP, whereas 3- and 15-acetyl DON are not affected by the imprinting (data not shown).

In a next step EDMA was applied, which is a commonly used cross-

linking monomer. The performance of MIPs was tested in aqueous media first, as they are intended to be operated as stationary phases in solid-phase extraction applications from aqueous matrices such as beer.

As it is the case with most MIPs generated with organic porogens, like acetonitrile, toluol or dichlormethane, they show significantly reduced binding capacities in solvents containing water. In general, it was observed that the imprinting effect is decreasing the more the water content increases. At 10 % water a separation could be obtained, which was significantly better than the control polymer. At water levels higher than 20 % the imprinting effect disappears and there is even no more separation of the acetone peak from DON. Neither is there a detectable separation of structural analogues. The imprinting effect in water is better restored by DON-4VP imprinted with 4-VP as functional monomer, than with MAA as functional monomer by DON-MAA (data not shown).

Using acetonitrile as the mobile phase, deoxynivalenol shows higher retention times than the structural very similar substances 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol and fusarenon-X. Whereas both control polymers (DON-4VP-BP and DON-MAA-BP) have difficulties in separating a mixture of DON, 3-acetyl DON and fusarenon-X, the same polymers imprinted with DON clearly result in distinctive peaks (see Figure 3). The retention index (DON-MAA and DON-MAA-BP) is 1.4 times higher than in the control polymer (1.3 in DON-4VP and DON-4VP-BP).

Table 3. DON-4VP and DON-MAA. The characterisation of both imprinted polymers including control polymers is listed below. Qualification was determined by investigating the separation of DON and structural analogues using HPLC columns filled with the

MIPs or the control polymers, respectively.

	log kow-value	CTL	kCTL	MIP	kMIP	aCTL	aMIP	RI-value
DON-4VP								
Acetone	-	7.03	-	7.14	-	-	-	-
DON	-0.71	9.98	0.4	13.08	0.8	1.0	1.0	1.00
Nivalenol	-2.22	12.6	0.8	17.44	1.4	0.5	0.6	0.92
Fusarenon-X	-1.24	9.07	0.3	9.93	0.4	1.4	2.1	0.68
15-acetyl DON	0.30	8.32	0.2	8.87	0.2	2.3	3.4	0.67
3-acetyl DON	-0.55	8.23	0.2	8.64	0.2	2.5	4.0	0.62
DON-MAA								
Acetone	-	6.9	-	7.0	-	-	-	-
DON	-0.71	8.6	0.2	11.6	0.7	1.0	1.0	1.00
Nivalenol	-2.22	9.0	0.3	12.6	0.8	0.8	0.8	0.98
Fusarenon-X	-1.24	7.6	0.1	8.1	0.2	2.4	4.2	0.58
15-acetyl DON	0.30	7.6	0.1	8.0	0.1	2.3	4.5	0.51
3-acetyl DON	-0.55	7.4	0.1	7.7	0.1	3.4	6.6	0.52

Conditions of HPLC runs: flow rate 0.5ml/min, back pressure of HPLC columns (250 x 4.6mm ID) 8bar

Successful imprinting is additionally characterised by the tailing effect evident in Figure 3. This tailing effect is explained by the distribution of binding sites with affinity constants of varying strength; each kind of these binding sites has its own binding site population.

As the capacity factors of the analytes are higher for the MIPs than for the control polymer, the DON imprinted polymer shows a markedly stronger affinity for each analyte (see Table 3). This effect is most pronounced for DON-MAA (k'MIP=0.7, k'CP=0.2 in organic mobile phase). The retention indices for three of the four other compounds are much less than one. Hence, the polymer is considered to be selective towards the initial template molecule. Only nivalenol shows a high cross-reactivity (RI-values of 0.98 and 0.92). This can be explained by the fact that DON has three different hydroxyl groups in 3, 7, and 15 position of the ring system. Whereas the OH-group in position 7 is non-reactive, the other two OH-groups are reactive and, therefore, im-

portant for the imprinting process. If these OH-groups do not exist, as it is the case in 15-acetyl and 3-acetyl DON, the recognition ability is decreased, explaining the low cross-reactivity to DON. In the control polymers DON-4VP-BP and DON-MAA-BP nivalenol shows a higher retention time than DON most likely due to its higher hydrophobicity.

HPLC results for zearalenone.

Imprinting of ZON was approached by conventionally using ZON in a 4-VP / EDMA co-polymer. Whereas the acetone peak shifts from 2.0 min in the blank polymer (ZON-4VP-BP) to 2.2 min in the imprinted polymer (ZON-4VP), the ZON peak shifts from 4.3 min to 5.4 min in the imprinted polymer (ZON-4VP and ZON-4VP-BP). The respective peak (see Figure 4 (a+b)) is tailed, indicating an imprinting effect: in both, the blank polymer and the MIP, the same ZON concentration has been applied. Results switching to the functional monomer TFM are worse (ZON-TFM and ZON-TFM-BP). A potential explanation for these problems seem to be related to the structure of ZON itself. ZON has a double bond incorporated in its 15en ring system. During polymerisation this reactive double bond could be responsible for an incorporation of ZON in the subsequent polymerisation process between the functional monomer and the cross-linker. Therefore, the ZON template molecules cannot be eluted from the generated imprinted polymer, thus blocking the binding sites and resulting in a low binding capacity. Another factor adversely influencing successful imprinting can be attributed to the large ring structure. As 3-dimensional simulations of ZON show this ring system is very flexible and will not remain in a defined sterical position during polymerisation. Due to this permanent movement the formation on an exactly fitting binding site is affected during the non-covalent pre-polymerisation step. Thus created binding sites will exhibit significant structural differences, which will decrease binding strength and selectivity of the synthesized MIP matrix.

Selection of a different template molecule without double bond, e.g. zearalanol, could avoid that problem. However, high material costs for ZON related substances have to be considered.

A cost-effective, yet less preferred alternative is based on using a structurally related compound during the imprinting procedure ('dummy' imprinting approach), which enables the formation of higher quality binding site. By comparison of a multitude of potential compounds substantial structural similarities in particular to quercetin (3,3´,4´,5,7 pentahydroxyflavone) become evident. Both substances have hydroxyl groups, attached to the aromatic ring system, as shown in Figure 2. However, since "similarity" is not "identity", the "dummy" approach is, as outlined above, less preferred compared to the direct impriting technology, mainly due to specificity reasons which are paramount, especially in trace analysis and trace removals.

Quercetin is a member of the subclass of the flavonols which are secondary metabolites of various plants, contributing to sensory properties, flavour and to the texture of fruits. Electron delocalisation throughout the whole ring system of quercetin is responsible for the antioxidant activity of quercetin and results from the formation of stable radicals (Russo et al. 2000). The anti-quercetin MIP shows high retention of quercetin. Polymers were successfully imprinted against quercetin and some structural analogues like morin. Zearalenone (ZON) is less similar to quercetin than other flavone compounds, hence less selective binding and retardation in an anti-quercetin MIP is expected. With increasing acetic acid concentration, the difference between the imprinted polymer Que-4VP and the blank polymer (Que-4VP-BP) for the retention of ZON decreases. However, selective retardation of ZON in a quercetin-imprinted polymer is evident in Figure 4 (c+d). The retention time of ZON in acetonitrile (0.1%HAc) as the mobile phase is increased by almost 50 % (from 8.6 min to 12.1 min) using the imprinted polymer as stationary phase in the HPLC experiment. The separation factor a was determined to be 1.64 and is therefore significantly better than the previously generated ZON-4VP polymer using ZON as the template molecule.

2.3. CONCLUSION

The obtained imprinting effects for the mycotoxin compounds

deoxynivalenol and zearalenone appear to be highly promising for the generation of synthetic receptor membranes. At this point of research, in particular DON-MAA with methacrylic acid as functional monomer seems to have the greatest potential due to the H-binding capabilities of MAA to the OH-groups of DON molecules. Further optimisation of the imprinting protocol for improving the binding capacity and binding strength and the transformance of the present results to other mycotoxins is with the teachings of the present specification well at hand for the skilled man in the art.

The development of such versatile synthetic receptors applicable for the surveillance and quality control of grains and beverages - in particular beer - represents a significant step forward in characterising mycotoxin contamination of food in general. Molecularly imprinted polymers can be stored at room temperature for years without loss in recognition power, they are thermally, mechanically and chemically robust and can be produced with standard laboratory equipment very cost effective. These factors are of particular interest considering ongoing global efforts towards regulating and controlling mycotoxin contamination in food and beverages. Hence, synthetic receptors based on molecularly imprinted polymers can be considered a competitive alternative to conventional antibodies used in affinity columns or sorbent assays in the near future.

Preferred technical features which were specifically responsible for successfully imprinting mykotoxins are:
For DON: the use of methacrylic acid and 4-Vinylpyridin as functional monomers instead of N,N-diethyl-2-aminoethylmethacrylate (DAEMA) and ethylenglykoldimethacrylate (EDMA) as crosslinker instead of divinylbenzene (DVB). This resulted in a significant increase of the dRT value from 0,7 to 3,1.

For ZON: Use of the "dummy template" quercetin instead of zearalenone resulting in an increase of dRT value from 0,7 to 3,4. Such dRT value increase was especially surprising, since up to date only the use of dummy templates from the same family of substances has been described (Matsui et al., 2000). In the present case, the dummy template is a member of the flavonoide

family (quercetin), which is not related at all to the mycotoxin zearalenone.

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Claims:

1. Method for binding mycotoxins to a solid carrier, comprising the following steps:
- contacting a mycotoxin containing solution, suspension or aerosol with a mycotoxin imprinted polymer,
- separating the bound mycotoxin from said solution, suspension or aerosol and
- optionally separating the mycotoxin from said polymer.

2. Method according to claim 1 characterized in that the mycotoxin imprinted polymer is specifically imprinted to a template selected from the group comprising calonectrin, deacetylcalonectrin, 7alpha,8alpha-dihydroxy-calonectrin, 7-hydroxycalonectrin, 8-hydroxy-calonectrin, 3-acetyldeoxy-nevalenol, 15-acetoxydeoxy-nivalenol, 3-acetyl-4,7-deoxynivalen, 3,15-diacetyl-deoxynivalenol, 4,7-dideoxynivalenol, deoxynivalenol, fusarenon-X, nivalenol, diacetylnivalenol, 4-acetyl-scirendiol, diepoxy-diacetyl-scirpenol, 4,15-diacetylscirpendiol, 3,4-diacetylscirpenol, 3,15-diacetylscirpenol, 4,15-diacetylscirpenol, 4,15 -diacetylscirpentriol, 7-hydroxy-isotrichodermin, 8-hydroxy-isotrichodermin, isotrichodermin, 3-monoacetylscirpenol, 15-monoacetylscirpenol, 8-oxodiacetylscirpenol, scirpentriol, triacetylscirpen, 15-acetyl-T-2-tetraol, acetyl T-2 toxin, 3,15-diacetyl-7,8-dihydroxy-12,13-epoxytrichotec-9-en, HT-2, 3'-hydroxy-HT-2, 3-hydroxytrichothecen, 3'-hydroxy-T-2-triol, neosolaniol, neosolaniol-monoacetat, NT-1 toxin, NT-2 toxin, 4-propanoyl HT-2, sporotrichiol, 4,8,15-triacetylscirpendiol, T-2 tetraol, T-2 toxin, 6'8'-dihydroxyzearalen, 5-formylzearalenon, 3'-hydroxyzearalenon, 7'-dehydrozearalenon, 8'-epi-hydroxyzearalenon, 8'-hydroxyzearalenon, zearalanon, zearalanol, zearalenol - cis, alpha,beta,zearalenol - trans, zearalenon-F2, butenolid, FS-1, FS-2, fusarin C, fusariocin C, fusarochromanon, 4-ipomeanol, moniliformin, poaefusarin, sporofusarin, trichotriol, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, aflatoxin M2, fumonisin B1, fumonisin B2, fumonisin B3, metabolites + derivatives or mixtures thereof.

3. Method according to claim 1 or 2, characterised in that said mycotoxin containing solution, suspension or aerosol is selected

from the group comprising field crop preparations, food preparations, water preparations, especially waste water, rinsing water, food processing water, environmental or industrial water samples or air samples.

4. Method according to any one of claims 1 to 3, characterised in that said polymers are based on functional monomers selected from the group comprising 4-vinylpyridines (4-VP), methacrylic acids (MAA), 2-trifluoromethylacrylic acids (TFM), methylmethacrylates or other alkylmethacrylates, alkylacrylates, allyl or arylacrylates and methacrylate, cyanoacrylate, styrene, alpha-methylstyrene, vinylester, especially vinylacetate, vinylchloride, methylvinylketon, vinylidenchloride, acrylamide, methacrylamid, acrylonitril, methacrylonitril, 2-acetamidoacrylic acid, 2-(acetoxyacetoxy)ethylmethacrylate, 1-acetoxy-1,3-butadiene, 2-acetoxy-3-butenenitrile, 4-acetoxystyrene, acrolein, acroleindiethylacetal, acroleindimethylacetal, acrylamide, 2-acrylamidoglycolic acid, 2-acrylamido-2-methylpropansulfonic acid, acrylic acid, acrylanhydride, acrylonitrile, acryloylchloride, (R)-alpha-acryloxy-beta, beta'-dimethyl-g-butyrolactone, N-acryloxysuccinimide N-acryloxytri.s(hydroxymethyl) aminomethane, N-acryloylchloride, N-acryloylpyrrolidone, N-acryloyl-tris(hydroxymethyl) amino methane, 2-amino ethyl methacrylate, N-(3-aminopropyl)methacrylamide, (o,m,or p)-amino-styrene, t-amylmethacrylate, 2-(1-aziridinyl)ethylmethacrylate, 2,2'-azobis-(2-amidinopropane), 2,2'-azobisisobutyronitrile, 4,4'-azobis-(4-cyanovaleric acid), 1,1'-azobis-(cyclohexanecarbonitrile);2,2'azobis-(2,4-dimethylvaleronitrile); 4-benzyloxy-3-methoxystyrene;2-bromoacrylic acid; 4-bromo-1-butene; 3-bromo-3,3-difluoropropane; 6-bromo-1-hexene; 3-bromo-2-methacrylonitrile: 2-(bromomethyl)acrylic acid; 8-bromo-1-octene; 5-bromo-1-pentene; cis-1-bromo-1-propene;.beta.-bromostyrene;p-bromostyrene, bromotrifluoro ethylene; (+/-)-3-buten-2-ol;1,3-butadiene;1,3-butadiene-1,4-dicarboxylic acid; 3-butenal diethyl acetal; 1-butene; 3-buten-2-ol; 3-butenyl chloroformate; 2-butylacrolein; N-t-butylacrylamide;butyl acrylate; butyl methacrylate; (o,m,p)-bromostyrene; t-butyl acrylate; (R)-carvone; (S)-carvone; (-)-carvyl acetate; cis 3-chloroacrylic acid; 2-chloroacrylonitrile; 2-chloroethyl vinyl ether; 2-chloromethyl-3-trimethylsilyl-1-

propene; 3-chloro-1-butene; 3-chloro-2-chloromethyl-1-propene; 3-chloro-2-methyl propene; 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; 3-chloro-1-phenyl-1-propene; m-chlorostyrene; o-chlorostyrene; p-chlorostyrene; 1-cyanovinyl acetate; 1-cyclopropyl-1-(trimethylsiloxy)ethylene; 2,3-dichloro-1-propene; 2,6-dichlorostyrene; 1,3-dichloropropene; 2,4-diethyl-2,6-heptadienal; 1,9-decadiene; 1-decene; 1,2-dibromoethylene; 1,1-dichloro-2,2-difluoroethylene; 1,1-dichloropropene; 2,6-difluorostyrene; dihydrocarveol; (+/-)-dihydrocarvone; (-)-dihydrocarvyl acetate; 3,3-dimethylacrylaldehyde; N,N'-dimethylacrylamide; 3,3-dimethylacrylic acid; 3,3-dimethylacryloyl chloride; 2,3-dimethyl-1-butene; 3,3-dimethyl-1-butene; 2-dimethyl aminoethyl methacrylate; 2,4-dimethyl-2,6-heptadien-1-ol; 2,4-dimethyl-2,6-heptadienal; 2,5-dimethyl-1,5-hexadiene; 2,4-dimethyl-1,3-pentadiene; 2,2-dimethyl-4-pentenal; 2,4-dimethylstyrene; 2,5-dimethylstyrene; 3,4-dimethylstyrene; divinyl benzene; divinyltetramethyl disiloxane; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-porphine; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-propionic acid; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-propionic acid disodium salt; 3,9-divinyl-2,4,8,10-tetraoraspiro[5,5]undecane; divinyl tin dichloride; 1-dodecene; 3,4-epoxy-1-butene; 2-ethyl acrolein; ethyl acrylate; 2-ethyl-1-butene; (+/-)-2-ethylhexyl acrylate; (+/-)-2-ethylhexyl methacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol triacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate; ethyl methacrylate; ethyl vinyl ether; ethyl vinyl ketone; ethyl vinyl sulfone; (1-ethylvinyl)tributyl tin; m-fluorostyrene; o-fluorostyrene; p-fluorostyrene; glycol metacrylate (hydroxyethyl methacrylate); GA GMA; 1,6-heptadiene; 1,6-heptadienoic acid; 1,6-heptadien-4-ol; 1-heptene; 1-hexen-3-ol; 1-hexene; hexafluoropropene; 1,6-hexanediol diacrylate; 1-hexadecene; 1,5-hexadien-3,4-diol; 1,4-hexadiene; 1,5-hexadien-3-ol; 1,3,5-hexatriene; 5-hexen-1,2-diol; 5-hexen-1-ol; hydroxypropyl acrylate; 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene; isoamyl methacrylate; isobutyl methacrylate; isoprene; 2-isopropenylaniline; isopropenyl chloroformate; 4,4'-isopropylidene dimethacrylate; 3-isopropyl-alpha-alpha-dimethylbenzene isocyanate; isopulegol; itaconic acid; itaconalyl chloride; lead(II)acrylate; (+/-)-linalool; linalyl acetate; p-mentha-1,8-diene; p-mentha-6,8-diene-2-ol; methyleneamino acetonitrile;

methacrolein; [3-(methacryloylamino)-propyl]trimethylammonium chloride; methacrylamide; methacrylic acid; methacrylic anhydride; methacrylonitrile; methacryloyl chloride; 2-(methacryloyloxy)ethyl acetoacetate; (3-methacryloxypropyl)trimethoxy silane; 2-(methacryloxy)ethyl trimethyl ammonium methylsulfate; 2-methoxy propene (isopropenyl methyl ether; methyl-2-(bromomethyl)acrylate; 5-methyl-5-hexen-2-one; methyl methacrylate; N,N'-methylene bisacrylamide; 2-methylene glutaronitrile; 2-methylene-1,3-propanediol; 3-methyl-1,2,-butadiene; 2-methyl-1-butene; 3-methyl-1-butene; 3-methyl-1-buten-1-ol; 2-methyl-1-buten-3-yne; 2-methyl-1,5-heptadiene; 2-methyl-1-heptene; 2-methyl-1-hexene; 3-methyl-1,3-pentadiene; 2-methyl-1,4-pentadiene; (+/-)-3-methyl-1-pentene; (+/-)-4-methyl-1-pentene; (+/-)-3-methyl-1-penten-3-ol; 2-methyl-1-pentene; .alpha.-methyl styrene; t-alpha-methylstyrene; t-beta.-methylstyrene; 3-methylstyrene; methyl vinyl ether; methyl vinyl ketone; methyl-2-vinyloxirane; 4-methylstyrene; methyl vinyl sulfone; 4-methyl-15-vinylthiazole; myrcene; t.beta.-nitrostyrene; 3-nitrostyrene; 1-nonadecene; 1,8-nonadiene; 1-octadecene; 1,7-octadiene; 7-octene-1,2-diol; 1-octene; 1-octen-3-ol; 1-pentadecene; 1-pentene; 1-penten-3-ol; t-2,4-pentenoic acid; 1,3-pentadiene; 1,4-pentadiene; 1,4-pentadien-3-ol; 4-penten-1-ol; 4-penten-2-ol; 4-phenyl-1-butene; phenyl vinyl sulfide; phenyl vinyl sulfonate; 2-propene-1-sulfonic acid sodium salt; phenyl vinyl sulfoxide; 1-phenyl-1-(trimethylsiloxy)ethylene; propene; salfrole; styrene (vinyl benzene); 4-styrene sulfonic acid sodium salt; Styrene sulfonyl chloride; 3-sulfopropyl acrylate potassium salt; 3-sulfopropyl methacrylate sodium salt; tetrachloroethylene; tetracyano ethylene; tetramethyldivinyl siloxane; trans 3-chloroacrylic acid; 2-trifluoromethyl propene; 2-(trifluoromethyl)propenoic acid; 2,4,4'-trimethyl-1-pentene; 3,5-bis(trifluoromethyl)styrene; 2,3-bis(trimethylsiloxy)-1,3-butadiene; 1-undecene; vinyl acetate; vinyl acetic acid; 4-vinyl anisole; 9-vinyl anthracene; vinyl behenate; vinyl benzoate; vinyl benzyl acetate; vinyl benzyl alcohol; 3-vinyl benzyl chloride; 3-(vinyl benzyl)-2-chloroethyl sulfone; 4-(vinyl benzyl)-2-chloroethyl sulfone; N-(p-vinyl benzyl)-N,N'-dimethyl amine; 4-vinyl biphenyl (4-phenyl styrene); vinyl bromide; 2-vinyl butane; vinyl butyl ether; 9-vinyl carbazole; vinyl carbinol; vinyl cetyl ether; vinyl

chloroacetate; vinyl chloroformate; vinyl crotanoate; vinyl cyclohexane; 4-vinyl-1-cyclohexene; 4-vinylcyclohexene dioxide; vinyl cyclopentene; vinyl dimethylchlorosilane; vinyl dimethylethoxysilane; vinyl diphenylphosphine; vinyl 2-ethyl hexanoate; vinyl 2-ethylhexyl ether; vinyl ether ketone; vinyl ethylene; vinyl ethylene iron tricarbonyl; vinyl ferrocene; vinyl formate; vinyl hexadecyl ether; vinylidene fluoride; 1-vinyl imidizole; vinyl iodide; vinyl laurate; vinyl magnesium bromide;vinyl mesitylene; vinyl 2-methoxy ethyl ether; vinyl methyl dichlorosilane; vinyl methyl ether; vinyl methyl ketone; 2-vinyl naphthalene; 5-vinyl-2-norbornene; vinyl pelargonate; vinyl phenyl acetate; vinyl phosphonic acid, bis(2-chloroethyl)ester; vinyl propionate; 4-vinyl pyridine; 2-vinyl pyridine; 1-vinyl-2-pyrrolidinone; 2-vinyl quinoline; 1-vinyl silatrane; vinyl sulfone; vinyl sulfone (divinylsulfone); vinyl sulfonic acid sodium salt; o-vinyl toluene; p-vinyl toluene; vinyl triacetoxysilane; vinyl tributyl tin; vinyl trichloride; vinyl trichlorosilane; vinyl trichlorosilane (trichlorovinylsilane); vinyl triethoxysilane; vinyl triethylsilane; vinyl trifluoroacetate; vinyl trimethoxy silane; vinyl trimethyl nonylether; vinyl trimethyl silane; vinyl triphenylphosphonium bromide (triphenyl vinyl phosphonium bromide); vinyl tris-(2-methoxyethoxy)silane; vinyl 2-valerate; acrylate-terminated or otherwise unsaturated urethanes, carbonates, and epoxies, especially allyl diglycol carbonate, glycidyl acrylate, glycidyl methacrylate, allyl glycidyl ether, and 1,2-epoxy-3-allyl propane; or mixtures thereof.

5. Method for preparing a mycotoxin imprinted polymer comprising the following steps:

- providing a mycotoxin template with a functional monomer in combination with a porogenic solvent so that a prepolymerisation complex of said template with said functional monomer is formed,
- co-polymerising said functional monomer with a crosslinker so that a co-polymer is formed,
- removing said template from said formed co-polymer at least partially and
- optionally further processing said formed co-polymer by a size reduction method, especially by crushing, grounding, milling or combinations of such size reduction methods.

6. Method according to claim 5, characterised in that said template is selected from the group comprising calonectrin, deacetylcalonectrin, 7alpha,8alpha-dihydroxy-calonectrin, 7-hydroxycalonectrin, 8-hydroxy-calonectrin, 3-acetyldeoxy-nevalenol, 15-acetoxydeoxy-nivalenol, 3-acetyl-4,7-deoxynivalen, 3,15-diacetyl-deoxynivalenol, 4,7-dideoxynivalenol, deoxynivalenol, fusarenon-X, nivalenol, diacetylnivalenol, 4-acetyl-scirendiol, diepoxy-diacetyl-scirpenol, 4,15-diacetylscirpendiol, 3,4-diacetylscirpenol, 3,15-diacetylscirpenol, 4,15-diacetylscirpenol, 4,15 -diacetylscirpentriol, 7-hydroxy-isotrichodermin, 8-hydroxy-isotrichodermin, isotrichodermin, 3-monoacetylscirpenol, 15-monoacetylscirpenol, 8-oxodiacetylscirpenol, scirpentriol, triacetylscirpen, 15-acetyl-T-2-tetraol, acetyl T-2 toxin, 3,15-diacetyl-7,8-dihydroxy-12,13-epoxytrichotec-9-en, HT-2, 3'-hydroxy-HT-2, 3-hydroxytrichothecen, 3'-hydroxy-T-2-triol, neosolaniol, neosolaniol-monoacetat, NT-1 toxin, NT-2 toxin, 4-propanoyl HT-2, sporotrichiol, 4,8,15-triacetylscirpendiol, T-2 tetraol, T-2 toxin, 6'8'-dihydroxyzearalen, 5-formylzearalenon, 3'-hydroxyzearalenon, 7'-dehydrozearalenon, 8'-epi-hydroxyzearalenon, 8'-hydroxyzearalenon, zearalanon, zearalanol, zearalenol - cis, alpha, beta,zearalenol - trans, zearalenon-F2, butenolid, FS-1, FS-2, fusarin C, fusariocin C, fusarochromanon, 4-ipomeanol, moniliformin, poaefusarin, sporofusarin, trichotriol, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, aflatoxin M2, fumonisin B1, fumonisin B2, fumonisin B3, or mixtures thereof.

7. Method according to claims 5 or 6, characterised in that said functional monomer is elected from the group comprising 4-vinylpyridine (4-VP), methacrylic acid (MAA), 2-trifluoro-methylacrylic acid (TFM), methylmethacrylates or other alkyl-methacrylates, alkylacrylates, allyl or arylacrylates and methacrylate, cyanoacrylate, styrene, alpha-methylstyrene, vinylester, especially vinylacetate, vinylchloride, methylvinylketon, vinylidenchloride, acrylamide, methacrylamid, acrylonitril, methacrylonitril, 2-acetamidoacrylic acid, 2-(acetoxyacetoxy)ethylmethacrylate, 1-acetoxy-1,3-butadiene, 2-acetoxy-3-butenenitrile, 4-acetoxystyrene, acrolein, acroleindiethylacetal, acroleindimethylacetal, acrylamide, 2-acrylamidoglycolic acid, 2-acrylamido-2-methylpropansulfonic acid,

acrylic acid, acrylanhydride, acrylonitrile, acryloylchloride, (R)-alpha-acryloxy-beta, beta'-dimethyl-g-butyrolactone, N-acryloxysuccinimide N-acryloxytri.s(hydroxymethyl) aminomethane, N-acryloylchloride, N-acryloylpyrrolidone, N-acryloyl-tris(hydroxymethyl) amino methane, 2-amino ethyl methacrylate, N-(3-aminopropyl)methacrylamide, (o,m,or p)-amino-styrene, t-amylmethacrylate, 2-(1-aziridinyl)ethylmethacrylate, 2,2'-azobis-(2-amidinopropane), 2,2'-azobisisobutyronitrile, 4,4'-azobis-(4-cyanovaleric acid), 1,1'-azobis-(cyclohexanecarbonitrile);2,2'azobis-(2,4-dimethylvaleronitrile); 4-benzyloxy-3-methoxystyrene;2-bromoacrylic acid; 4-bromo-1-butene; 3-bromo-3,3-difluoropropane; 6-bromo-1-hexene; 3-bromo-2-methacrylonitrile: 2-(bromomethyl)acrylic acid; 8-bromo-1-octene; 5-bromo-1-pentene; cis-1-bromo-1-propene;.beta.-bromostyrene;p-bromostyrene, bromotrifluoro ethylene; (+/-)-3-buten-2-ol; 1,3-butadiene; 1,3-butadiene-1,4-dicarboxylic acid; 3-butenal diethyl acetal; 1-butene; 3-buten-2-ol; 3-butenyl chloroformate; 2-butylacrolein; N-t-butylacrylamide;butyl acrylate; butyl methacrylate; (o,m,p)-bromostyrene; t-butyl acrylate; (R)-carvone; (S)-carvone; (-)-carvyl acetate; cis 3-chloroacrylic acid; 2-chloroacrylonitrile; 2-chloroethyl vinyl ether; 2-chloromethyl-3-trimethylsilyl-1-propene; 3-chloro-1-butene; 3-chloro-2-chloromethyl-1-propene; 3-chloro-2-methyl propene; 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; 3-chloro-1-phenyl-1-propene; m-chlorostyrene; o-chlorostyrene, o-chlorostyrene; p-chlorostyrene; 1-cyanovinyl acetate; 1-cyclopropyl-1-(trimethylsiloxy)ethylene; 2,3-dichloro-1-propene; 2,6-dichlorostyrene; 1,3-dichloropropene; 2,4-diethyl-2,6-heptadienal; 1,9-decadiene; 1-decene; 1,2-dibromoethylene; 1,1-dichloro-2,2-difluoroethylene; 1,1-dichloropropene; 2,6-difluorostyrene; dihydrocarveol; (+/-)-dihydrocarvone; (-)-dihydrocarvyl acetate; 3,3-dimethylacrylaldehyde; N,N'-dimethylacrylamide; 3,3-dimethylacrylic acid; 3,3-dimethylacryloyl chloride; 2,3-dimethyl-1-butene; 3,3-dimethyl-1-butene; 2-dimethyl aminoethyl methacrylate; 2,4-dimethyl-2,6-heptadien-1-ol; 2,4-dimethyl-2,6-heptadienal; 2,5-dimethyl-1,5-hexadiene; 2,4-dimethyl-1,3-pentadiene; 2,2-dimethyl-4-pentenal; 2,4-dimethylstyrene; 2,5-dimethylstyrene; 3,4-dimethylstyrene; divinyl benzene; divinyltetramethyl disiloxane; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-porphine; 8,13-divinyl-3,7,12,17-

tetramethyl-21H,23H-propionic acid; 8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-propionic acid disodium salt; 3,9-divinyl-2,4,8,10-tetraoraspiro[5,5]undecane; divinyl tin dichloride; 1-dodecene; 3,4-epoxy-1-butene; 2-ethyl acrolein; ethyl acrylate; 2-ethyl-1-butene; (+/-)-2-ethylhexyl acrylate; (+/-)-2-ethylhexyl methacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol triacrylate; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate; ethyl methacrylate; ethyl vinyl ether; ethyl vinyl ketone; ethyl vinyl sulfone; (1-ethylvinyl)tributyl tin; m-fluorostyrene; o-fluorostyrene; p-fluorostyrene; glycol metacrylate (hydroxyethyl methacrylate); GA GMA; 1,6-heptadiene; 1,6-heptadienoic acid; 1,6-heptadien-4-ol; 1-heptene; 1-hexn-3-ol; 1-hexene; hexafluoropropene; 1,6-hexanediol diacrylate; 1-hexadecene; 1,5-hexadien-3,4-diol; 1,4-hexadiene; 1,5-hexadien-3-ol; 1,3,5-hexatriene; 5-hexen-1,2-diol; 5-hexen-1-ol; hydroxypropyl acrylate; 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene; isoamyl methacrylate; isobutyl methacrylate; isoprene; 2-isopropenylaniline; isopropenyl chloroformate; 4,4'-isopropylidene dimethacrylate; 3-isopropyl-a-a-dimethylbenzene isocyanate; isopulegol; itaconic acid; itaconalyl chloride; lead(II)acrylate; (+/-)-linalool; linalyl acetate; p-mentha-1,8-diene; p-mentha-6,8-diene-2-ol; methyleneamino acetonitrile; methacrolein; [3-(methacryloylamino)-propyl]trimethylammonium chloride; methacrylamide; methacrylic acid; methacrylic anhydride; methacrylonitrile; methacryloyl chloride; 2-(methacryloyloxy)ethyl acetoacetate; (3-methacryloxypropyl)trimethoxy silane; 2-(methacryloxy)ethyl trimethyl ammonium methylsulfate; 2-methoxy propene (isopropenyl methyl ether; methyl-2-(bromomethyl)acrylate; 5-methyl-5-hexen-2-one; methyl methacrylate; N,N'-methylene bisacrylamide; 2-methylene glutaronitrile; 2-methylene-1,3-propanediol; 3-methyl-1,2,-butadiene; 2-methyl-1-butene; 3-methyl-1-butene; 3-methyl-1-buten-1-ol; 2-methyl-1-buten-3-yne; 2-methyl-1,5-heptadiene; 2-methyl-1-heptene; 2-methyl-1-hexene; 3-methyl-1,3-pentadiene; 2-methyl-1,4-pentadiene; (+/-)-3-methyl-1-pentene; (+/-)-4-methyl-1-pentene; (+/-)-3-methyl-1-penten-3-ol; 2-methyl-1-pentene; .alpha.-methyl styrene; t-a-methylstyrene; t-beta.-methylstyrene; 3-methylstyrene; methyl vinyl ether; methyl vinyl ketone; methyl-2-vinyloxirane; 4-methylstyrene; methyl vinyl sulfonee; 4-methyl-15-vinylthiazole; myrcene; t.beta.-ni-

trostyrene; 3-nitrostyrene; 1-nonadecene; 1,8-nonadiene; 1-octadecene; 1,7-octadiene; 7-octene-1,2-diol; 1-octene; 1-octen-3-ol; 1-pentadecene; 1-pentene; 1-penten-3-ol; t-2,4-pentenoic acid; 1,3-pentadiene; 1,4-pentadiene; 1,4-pentadien-3-ol; 4-penten-1-ol; 4-penten-2-ol; 4-phenyl-1-butene; phenyl vinyl sulfide; phenyl vinyl sulfonate; 2-propene-1-sulfonic acid sodium salt; phenyl vinyl sulfoxide; 1-phenyl-1-(trimethylsiloxy)ethylene; propene; salfrole; styrene (vinyl benzene); 4-styrene sulfonic acid sodium salt; Styrene sulfonyl chloride; 3-sulfopropyl acrylate potassium salt; 3-sulfopropyl methacrylate sodium salt; tetrachloroethylene; tetracyano ethylene; tetramethyldivinyl siloxane; trans 3-chloroacrylic acid; 2-trifluoromethyl propene; 2-(trifluoromethyl)propenoic acid; 2,4,4'-trimethyl-1-pentene; 3,5-bis(trifluoromethyl)styrene; 2,3-bis(trimethylsiloxy)-1,3-butadiene; 1-undecene; vinyl acetate; vinyl acetic acid; 4-vinyl anisole; 9-vinyl anthracene; vinyl behenate; vinyl benzoate; vinyl benzyl acetate; vinyl benzyl alcohol; 3-vinyl benzyl chloride; 3-(vinyl benzyl)-2-chloroethyl sulfone; 4-(vinyl benzyl)-2-chloroethyl sulfone; N-(p-vinyl benzyl)-N,N'-dimethyl amine; 4-vinyl biphenyl (4-phenyl styrene); vinyl bromide; 2-vinyl butane; vinyl butyl ether; 9-vinyl carbazole; vinyl carbinol; vinyl cetyl ether; vinyl chloroacetate; vinyl chloroformate; vinyl crotanoate; vinyl cyclohexane; 4-vinyl-1-cyclohexene; 4-vinylcyclohexene dioxide; vinyl cyclopentene; vinyl dimethylchlorosilane; vinyl dimethylethoxysilane; vinyl diphenylphosphine; vinyl 2-ethyl hexanoate; vinyl 2-ethylhexyl ether; vinyl ether ketone; vinyl ethylene; vinyl ethylene iron tricarbonyl; vinyl ferrocene; vinyl formate; vinyl hexadecyl ether; vinylidene fluoride; 1-vinyl imidizole; vinyl iodide; vinyl laurate; vinyl magnesium bromide;vinyl mesitylene; vinyl 2-methoxy ethyl ether; vinyl methyl dichlorosilane; vinyl methyl ether; vinyl methyl ketone; 2-vinyl naphthalene; 5-vinyl-2-norbornene; vinyl pelargonate; vinyl phenyl acetate; vinyl phosphonic acid, bis(2-chloroethyl)ester; vinyl propionate; 4-vinyl pyridine; 2-vinyl pyridine; 1-vinyl-2-pyrrolidinone; 2-vinyl quinoline; 1-vinyl silatrane; vinyl sulfone; vinyl sulfone (divinylsulfone); vinyl sulfonic acid sodium salt; o-vinyl toluene; p-vinyl toluene; vinyl triacetoxysilane; vinyl tributyl tin; vinyl trichloride; vinyl trichlorosilane; vinyl trichlorosilane (trichlorovinylsilane); vinyl trieth-

oxysilane; vinyl triethylsilane; vinyl trifluoroacetate; vinyl trimethoxy silane; vinyl trimethyl nonylether; vinyl trimethyl silane; vinyl triphenylphosphonium bromide (triphenyl vinyl phosphonium bromide); vinyl tris-(2-methoxyethoxy)silane; vinyl 2-valerate; acrylate-terminated or otherwise unsaturated urethanes, carbonates, and epoxies, especially allyl diglycol carbonate, glycidyl acrylate, glycidyl methacrylate, allyl glycidyl ether, and 1,2-epoxy-3-allyl propane; or mixtures thereof.

8. Method according to any one of claims 5 to 7, characterised in that said porogenic solvent is selected from the group comprising acetonitrile, methanol , acetone and other apolar solvents, benzene, toluene, chloroform, dichlormethane, tetrahydrofurane, dimethylformamide, dimethylsulfoxide, ethanol, 1-propanole, methanol, water or mixtures thereof.

9. Method according to any one of claims 5 to 8, characterised in that said crosslinker is selected from the group comprising di-, tri- and tetrafunctional acrylates or methacrylates, divinylbenzene (DVB), alkyleneglykols, polyalkyleneglycoldiacrylates and methacrylates, especially ethylenglycoldimethacrylate (EDMA), trimethyltrimeth-acrylate (TRIM), or ethylenglycoldiacrylate, vinyl or allylacrylates or methacrylates, diallyldiglycoldicarbonate, diallylmaleate, diallylfumarat, diallylitaconate, vinylesters, especially divinyloxalate, divinylmalonate, diallylsuccinate, triallylisocyanurate, dimethacrylates or diacrylates of bis-ophenol A or ethoxylated bis-phenol A, methylene or polymethylene bisacrylamide or bismethayrylamide, hexamethylenebisacrylamide or hexamethylenbismethacrylamide, di(alkene)tertiary amine, trimethylolpropantriacrylate, pentaerythritoltetraacrylate, divinylether, divinylsulfone, diallylphtalates, triallylmelamine, 2-isocyanatoethylmethacrylate, 2-isocyanatoethylacrylate, 3-isocyanatopropylacrylate, 1-methyl-2-isocyanatoethylmethacrylate, 1,1-dimethyl-2-isocyanaotoethylacrylate, tetraethyleneglycoldiacrylate, tetraethyleneglycoldimethacrylate, hexanedioldimethacrylate, hexanedioldiacrylate or mixtures thereof.

10. Method according to any one of claims 5 to 9, characterised

in that 2,2'-Azobis-isobutyronitrile (AIBN), 2,2'-azobis-(2,4-dimethylvaleronitrile) (ABDV) or mixtures thereof are used as starters for said co-polymerisation.

11. Method according to any one of claims 5 to 10, characterised in that said co-polymerisation is carried out in an oxygen-free atmosphere.

12. Method according to any one of claims 5 to 11, characterised in that said co-polymerisation is started by photoinitiation or by thermal initiation.

13. Mycotoxin imprinted polymers with a mycotoxin dRT value of more than 0,7.

14. Polymers according to claim 13 obtainable by a method according to any one of claims 5 to 12.

15. Polymers according to claim 13 or 14 with a mycotoxin dRT value of 1,0 or more, especially of 3,0 or more.

16. Polymer according to any one of claims 13 to 15, characterised in that it specifically binds to nivalenole, deoxynivalenole, zearalenone, T2 toxin, HT2 toxin, or mixtures thereof.

17. Polymer according to any one of claims 13 to 16, characterised in that it is built up by 4-vinylpyridine, methacrylic acid, 2-trifluoromethacrylic acid or mixtures thereof as functional monomers.

18. Use of mycotoxin imprinted polymers according to any one of claims 13 to 17 for solid phase extraction of mycoctoxins from mycotoxin containing solutions or suspensions.

19. Use of mycotoxin imprinted polymers according to any one of claims 13 to 17 as food additives.

20. Use of mycotoxin imprinted polymers according to any one of claims 13 to 17 for the preparation of a medicament for treating or preventing mycotoxin-caused disorders.

21. Use of mycotoxin imprinted polymers according to any one of claims 13 to 17 for diagnosing the presence of mycotoxins in a tissue sample or fluid sample from humans, from animals or from plants.

22. Use of mycotoxin imprinted polymers according to any one of claims 13 to 17 in a sensor for detecting the presence and/or amount of mycotoxins being present in a biological, environmental or industrial sample.

23. Use of mycotoxin imprinted polymers according to any one of claims 13 to 17 for cleaning of mycotoxin contaminated solutions, suspensions and aerosols.

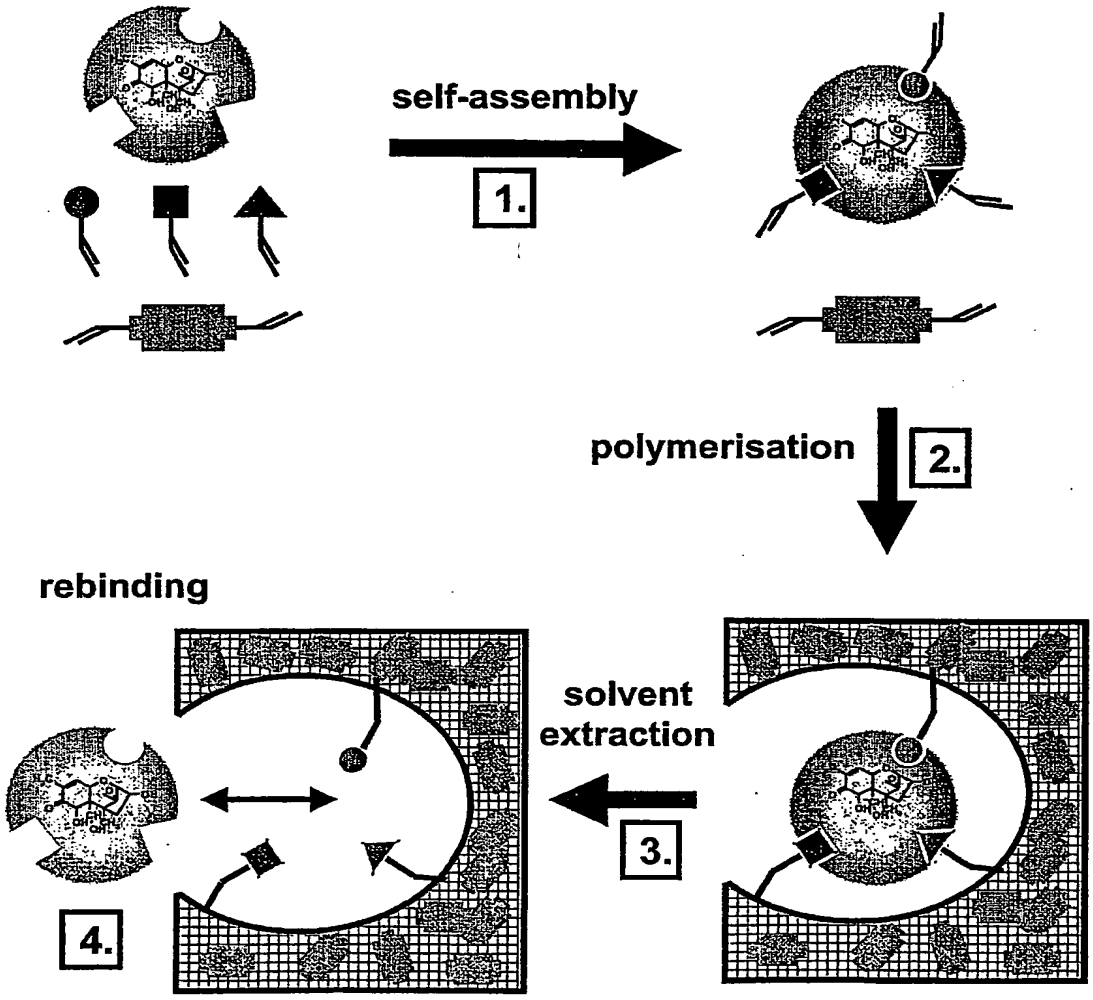
self-assembly
1.
polymerisation
2.
rebinding
solvent
extraction
3.
4.

FIG. 1

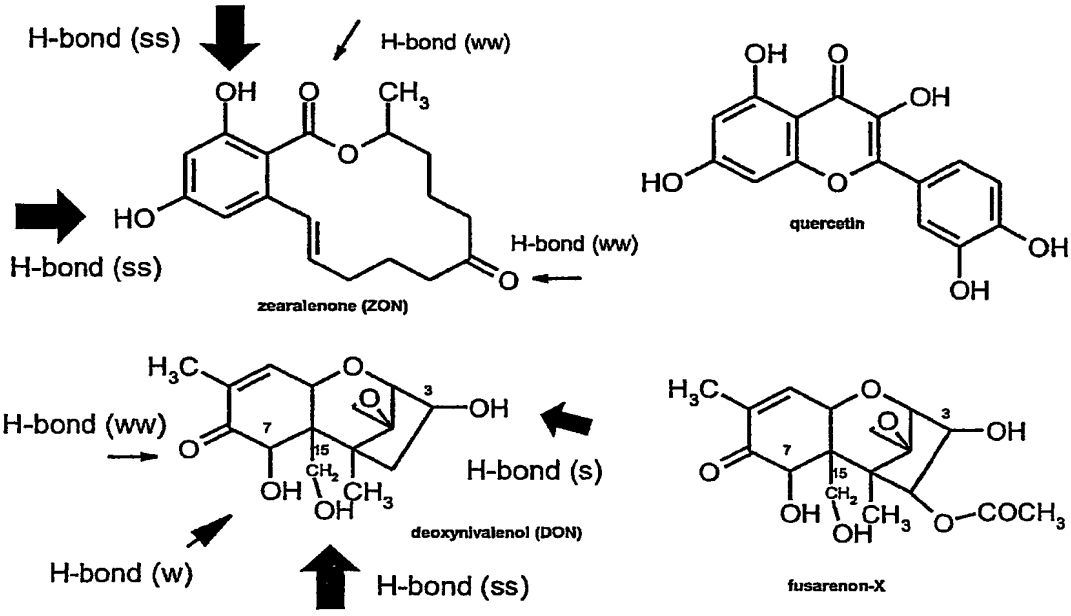
H-bond (ss)
H-bond (ww)
H-bond (ss)
H-bond (ww)
zearalenone (ZON)
quercetin
H-bond (ww)
H-bond (s)
H-bond (w)
H-bond (ss)
deoxynivalenol (DON)
fusarenon-X

FIG. 2

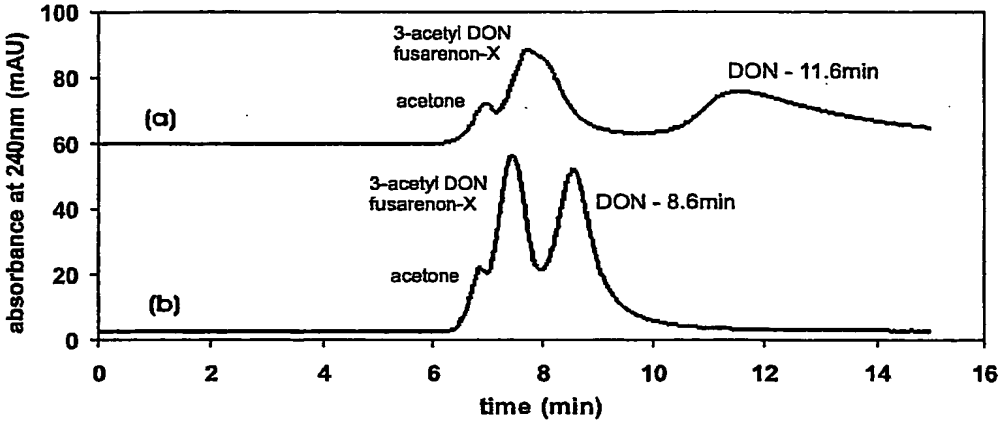
absorbance at 240nm (mAU)
100
80
60
40
20
0
3-acetyl DON
fusarenon-X
acetone
(a)
DON - 11.6min
3-acetyl DON
fusarenon-X
acetone
(b)
DON - 8.6min
0
2
4
6
8
10
12
14
16
time (min)

FIG. 3

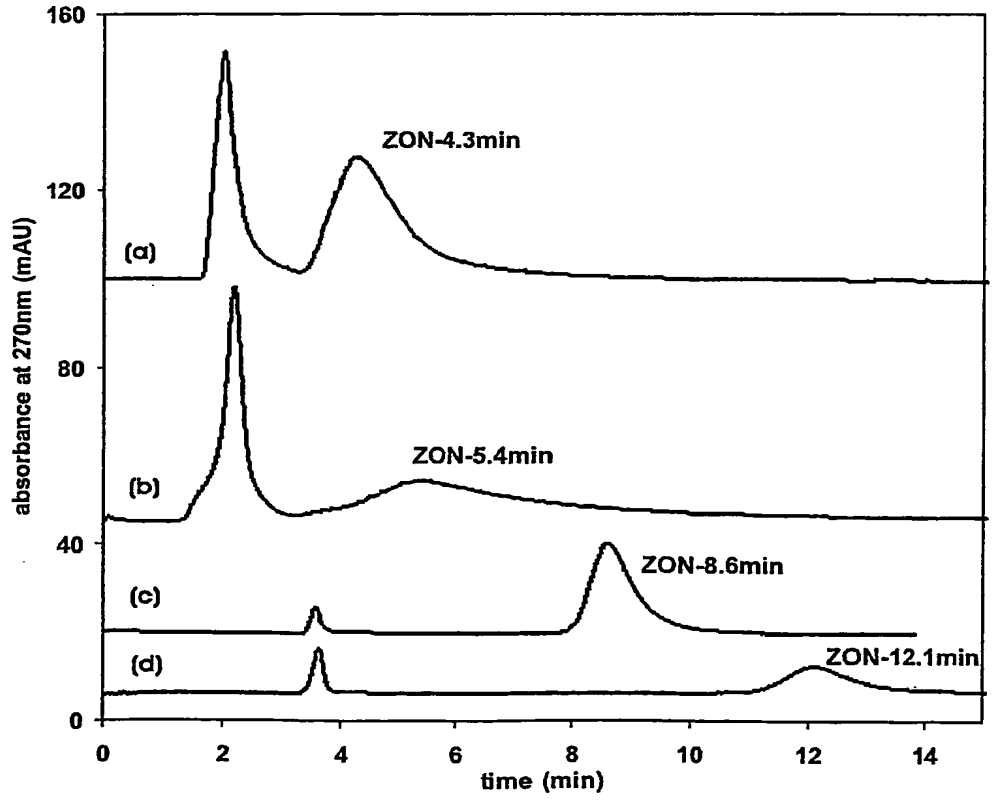
absorbance at 270nm (mAU)
160
120
80
40
0
(a)
ZON-4.3min
(b)
ZON-5.4min
(c)
ZON-8.6min
(d)
ZON-12.1min
0
2
4
6
8
10
12
14
time (min)

FIG. 4

INTERNATIONAL SEARCH REPORT

Int al Application No
PCT/EP 03/05688

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01D15/08 B01J20/26 A61K9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01D B01J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, MEDLINE, EPO-Internal, EMBASE, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ALEGAKIS A K ET AL: "Deactivation of mycotoxins. I. An in vitro study of zearalenone adsorption on new polymeric adsorbents." JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH PART B PESTICIDES FOOD, vol. 34, no. 4, July 1999 (1999-07), pages 633-644, XP009017963 July, 1999 ISSN: 0360-1234 the whole document	1-23

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 September 2003	10/10/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pinheiro Vieira, E

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JODLBAUER JUSTUS ET AL: "Towards ochratoxin A selective molecularly imprinted polymers for solid-phase extraction." JOURNAL OF CHROMATOGRAPHY. A. NETHERLANDS 1 FEB 2002, vol. 945, no. 1-2, 1 February 2002 (2002-02-01), pages 45-63, XP004333336 cited in the application the whole document	1-23
X	WEISS ROBERT ET AL: "Molecular imprinting and solid phase extraction of flavonoid compounds." BIOSEPARATION, vol. 10, no. 6, 2001, pages 379-387, XP009017962 ISSN: 0923-179X the whole document	1-23
Y	RAMSTROM OLOF ET AL: "Food analyses using molecularly imprinted polymers." JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 49, no. 5, May 2001 (2001-05), pages 2105-2114, XP002255748 ISSN: 0021-8561 cited in the application the whole document	1-23
Y	EP 0 038 792 A (SAGMEISTER FRANZ DR) 28 October 1981 (1981-10-28) the whole document	1-23
A	ANDERSSON L I: "Molecular imprinting: developments and applications in the analytical chemistry field" JOURNAL OF CHROMATOGRAPHY. BIOMEDICAL APPLICATIONS, ELSEVIER, AMSTERDAM, NL, vol. 745, no. 1, 4 August 2000 (2000-08-04), pages 3-13, XP004215285 ISSN: 0378-4347 the whole document	1-23

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INTERNATIONAL SEARCH REPORT

Int al Application No
PCT/EP 03/05688

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	WEISS R ET AL: "Improving methods of analysis for mycotoxins: Molecularly imprinted polymers for deoxynivalenol and zearalenone." FOOD ADDITIVES AND CONTAMINANTS, vol. 20, no. 4, 20 April 2003 (2003-04-20), pages 386-395, XP009017977 ISSN: 0265-203X the whole document	1-23

INTERNATIONAL SEARCH REPORT

Inte ıal Application No
PCT/EP 03/05688

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		AT 370946 B	10-05-1983
		AU 6965281 A	29-10-1981
		DE 3161160 D1	17-11-1983
		EP 0038792 A1	28-10-1981
		NZ 196709 A	30-11-1983
		ZA 8102675 A	26-05-1982